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POSTSHOCK PULMONARY PLATELET TRAPPING

An experimental study on animals



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Abstract Platelets are supposed to play a significant role in the development of postshock pulmonary insufficiency. During shock and during the direct postshock period platelets are trapped in the lungs. This mechanical plugging together with released vasoactive substances causes a changed pulmonary function, which is by many thought to be an important factor in the pathophysiology of the adult respiratory distress syndrome (ARDS). These experiments on animals were performed to study early postshock pulmonary platelet trapping (PPT) and to evaluate the effect of three different drugs, the effect of pre-shock denervation of one lung, and the effect of alcohol intoxication on PPT. Two shock models - traumatic and endotoxin shock - were used. In the first two experiments PPT was studied at a defined time after shock induction using ⁵¹Cr labeled platelets. PPT was significant following both endotoxin and traumatic shock. Pulmonary platelet sequestration was diminished with prostaglandin E1-treatment, but acetylsalicylic acid alone or in combination with prostaglandin had no effect on endotoxin induced PPT. Surgical denervation of the left lung, performed two months before shock induction, attenuated PPT in both shock models. Dynamic recording of PPT by continuous scanning of the lungs, using ¹¹¹In labeled platelets was also performed. This demonstrated PPT within three to five minutes following both endotoxin and traumatic shock. Pulmonary platelet trapping was significantly diminished following prostaglandin E1-treatment, but Ticlopidine had no effect on PPT in this dynamic model. Traumatic shock to pigs pretreated with alcohol resulted in a significantly increased PPT and increased pulmonary vascular resistance when compared to traumatized non-intoxicated pigs.			
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POSTSHOCK

PULMONARY PLATELET TRAPPING

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by

Johan Thörne

Lund 1985

The present thesis is based on the following papers:

- I Effects of Acetylsalicylic Acid and Prostaglandin on Endotoxin-induced Pulmonary Platelet Sequestration.
Johan Thörne, Marian Kuenzig and Seymour I Schwartz
Acta Chir Scand 147: 161-162, 1981
- II Effect of Denervation of a Lung on Pulmonary Platelet Trapping Associated with Traumatic Shock.
Johan Thörne, Marian Kuenzig, Homeira M McDonald, Seymour I Schwartz
Surgery 88: 208-213, 1980
- III An Experimental Method for In Vivo Studies of Pulmonary Platelet Sequestration
Johan Thörne, Per-Ingvar Olsson, Sven-Erik Strand, Bo Anders Jönsson and Lars Norgren
Eur J Nucl Med 9: 472-477, 1984
- IV In Vivo Study of Endotoxin Induced Pulmonary Platelet Sequestration. Effects of Two Inhibitory Drugs
Johan Thörne, Bo Anders Jönsson, Lars Norgren and Sven-Erik Strand
Submitted
- V Early Posttraumatic Pulmonary Platelet Trapping and Its Potentiation by Oral Pretreatment with Alcohol.
Johan Thörne, Sten Blomqvist, Olle Elmér, Bo Anders Jönsson, Sten Lindahl and Sven-Erik Strand
Submitted
- VI Early Posttraumatic Changes of Hemodynamics and Pulmonary Ventilation in Alcohol Pretreated Pigs
Sten Blomqvist, Johan Thörne, Olle Elmér, Bo Anders Jönsson, Sven-Erik Strand and Sten G E Lindahl
Submitted

These papers will be referred to in the text by their Roman numerals.

YOU MAN -
BE HUMAN

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ABBREVIATIONS

ARDS	adult respiratory distress syndrome
ASA	acetyl salicylic acid
C TOT	lung compliance
E'CO ₂	end tidal carbon dioxide concentration
h.r.	heart rate
MBq	megaBequerel
NPE	neurogenic pulmonary edema
Pa CO ₂	arterial carbondioxide pressure
Pa O ₂	arterial oxygen pressure
PAo	mean arterial pressure
PAP	mean pulmonary artery pressure
PCW	mean pulmonary capillary wedge pressure
PGE ₁	prostaglandin E ₁
PGI ₂	prostaglandin I ₂ (prostacyclin)
PPT	pulmonary platelet trapping
Pra	mean right atrial pressure

PVR	pulmonary vascular resistance
Q	cardiac output
ROI	regions of interest
SFP	screen filtration pressure
SV	stroke volume
SVR	systemic vascular resistance
SWL	stroke work left ventricle
SWR	stroke work right ventricle
TxA ₂	thromboxane
V _{CO2}	carbon dioxide elimination
V _E eff	effective minute ventilation

INTRODUCTION

Shock of varying etiology may under certain circumstances cause pulmonary insufficiency - adult respiratory distress syndrome (ARDS). This syndrome is characterized by interstitial pulmonary edema, progressive hypoxemia and later in the most serious cases, secondary to anoxia, death due to cardiac collapse (1, 2), The problem was recognized by Zenker 1862 (3) and the clinical syndrome was described by Jenkins et al. 1950 (4) but the real interest in respiratory failure following traumatic shock was accelerated first during and after the Vietnam war. During this period the syndrome was given a variety of denominations i. e. Vietnam lung, wet lung, Da Nang lung, certainly due to the confusion concerning the etiology of the syndrome. Despite different etiologic factors as sepsis, trauma, major surgery, they may all result in respiratory problems with the same clinical picture, depending on the response to the injury. A primary local response (edema, rubor, calor) is in the severe cases followed by a general effect - shock. To find the mechanism behind this response and the subsequent respiratory problem a large number of studies have been carried out.

Jenkins et al. (4) described three cases of pulmonary insufficiency due to trauma, all of them developing a clinical picture characterized by a sudden onset of dyspnoe, tachypnoe, expiratory effort, fever and hypotension. The condition was

aggravated by blood transfusion. This and similar findings gave rise to the idea that the syndrome was caused by transfusion of stored blood. Due to rapid resuscitation with fluid and blood transfusion a considerable increase in the number of survivors in the Vietnam war was achieved. This resulted however in new problems among the badly injured, for example respiratory failure. Despite the fact that the blood pressure, kidney function and cardiac function were rapidly normalized, making the shock period fairly short, respiratory failure was sometimes seen. Ashbaugh et al. described in 1967 (5) the syndrome in civilian non-thoracic trauma victims. They found a pathologic picture similar to the infant respiratory distress syndrome (IRDS) and therefore named the syndrome "adult respiratory distress syndrome" - ARDS.

Based on the assumption that abnormal coagulation after shock is an important factor the syndrome has also been called "the microembolism syndrome" (6). Following major soft tissue trauma or fractures, thromboplastic material is released from the damaged area, partly at the time of trauma and partly posttraumatically. Thromboplastin gives rise to the formation of thrombin and fibrin resulting in microemboli in the lungs.

This feared clinical syndrome is greatly a result of improved treatment of severely injured and/or septic patients, who earlier did not survive the primary trauma or infection. Still the exact mechanism of the initiation of the syndrome is unclear. ARDS is

described by Moore (2) as a 4-phased event where the 2 first phases consist of minor changes in the lungs which are reversible because of, or despite, the given treatment. The next two phases are considered irreversible, so early treatment (or prophylaxis) is at present the way to possible success.

It is well known that several conditions (major surgery, severe head injuries, long bone fractures, multiple trauma, burns, severe infections) may sometimes result in respiratory failure (7, 8, 9, 10) and despite several underlying causes the same clinical and pathologic picture develops.

The knowledge of the initial and causative mediators is of great importance for the early institution of adequate treatment.

Factors that have been discussed as being responsible for producing the changes in the lungs are oxygen toxicity (11), neurogenic factors (12), sepsis (13), humoral factors (14, 15, 16). Although multiple, probably all these factors are able to initiate reactions that finally result in ARDS.

In the early postshock period pulmonary platelet and leucocyte trapping in the lungs is probably important, as is the release of vasoactive substances, activation of the complement, coagulation and fibrinolytic systems, thus activation of a cascade of reactions, where the relative importance of each system is so far uncertain (17). Many experiments have been made on animals where

one or two of the above mentioned systems have been studied in an attempt to elucidate their importance in the development of ARDS.

Experimental models

Animal studies have led to the proposal that platelets, leucocytes, the complement system and humoral factors such as histamin, serotonin, prostaglandin etc. are mediators for the respiratory failure. Several studies both experimental and clinical have been made in order to clarify the problem. Clinical investigations on this subject are difficult - the monitoring of the patient is usually started late in the initial shock phase, when the primary reaction, that later can give rise to respiratory failure, has already started. The possible way to study very early stages in the reaction is to use animal models. Different experimental shock models producing acute respiratory distress have been described - soft tissue trauma (18, 19, 20, 21) missile injury (22), blood embolus (23), tourniquet shock (24, 25), oleic acid (26), endotoxin - septic shock (16, 27, 28, 29, 30, 31), tissue ischemia (32) and increased intracranial pressure (33, 34, 35). Several of these experimental models are of less value due to their complexity and unrealistic situations and they are sometimes difficult to reproduce.

Heideman et al. (16, 18) registered complement activation following soft tissue trauma and endotoxin shock. They also demonstrated that endotoxin shock caused an immediate increase

in pulmonary platelet trapping and that following soft tissue trauma there was a slow and gradual increase of platelet aggregation in the lungs during the postshock hours.

The two shock models used in these experiments - i. v. injection of endotoxin and soft tissue trauma according to Blalock (36) have been demonstrated to produce PPT. This is demonstrated in studies done by Bergentz et al. (37) and ourselves (120) following trauma in dog experiments. Among others (16, 31) Genell et al. (38) showed significant trapping of platelets in the lungs following endotoxin shock. The endotoxin shock model could be considered as being artificial, but the model is well documented, often used and found to have effects similar to clinical sepsis (10).

Heideman et al. (39) also produced shock reaction with reduction of circulating platelets and PPT following injection of muscle tissue. The pattern of response was similar to that seen after soft tissue trauma (18), but with a more sudden onset. Despite the similar reaction, the situation must be considered abnormal, and some steps in the very early reaction following trauma must be missed, making studies on early PPT and different drugs effect on PPT impossible.

Jansson et al. (19) demonstrated a good trauma model using missile injury. The method is probably easy to reproduce but seems to be less applicable to PPT studies in direct connection with the injury.

Tourniquet injuries to a hind limb in rats described by among others Robinson (40) resulted in decreased platelet count and microembolization to the lungs, but to a very moderate degree, suggesting a rather mild trauma. The blood pressure as an indication of shock was not measured. Similar studies confirming these results have been made by aortic clamping. Stallone et al. (32) demonstrated pulmonary changes (hemorrhage, emboli, edema) following 4 hours of aortic clamping.

Following 2 hours of aortic clamping Bowald et al. (41) demonstrated similar changes in lung biopsies, but PPT could not be shown by external measurements of ⁵¹Cr labeled platelets. The local ischemia during clamping produces acid metabolites which are washed out following declamping, and has been proposed as the cause of shock reaction. The model has clinical similarities, but for studies on acute pulmonary platelet trapping the injury is probably too extended in time and mild making variations in PPT difficult to analyse.

Bowald et al. (41) also demonstrated increased pulmonary capillary wedge pressure, interpreted as being caused by pulmonary microembolism. The early changes in pulmonary hemodynamic following shock are characterized by reduced PaO₂ and vasoconstriction causing increased pulmonary vascular resistance (PVR) (42, 43), but in this early phase without pulmonary edema. Increased PVR is experimentally induced by platelet aggregation following different causes of shock (27, 44). Some authors

propose that this is due to mechanical blockage of the pulmonary circulation (44) but most investigators believe that PVR is increased by vasoconstriction (45, 46). Thus there is no doubt that PVR is increased following shock but the question is why. Rådegran et al. - (47, 48) demonstrated that ASA treatment prevented increased PVR but did not inhibit PPT following thrombin infusion.

Platelets

Blaisdell (49) demonstrated in a clinical study that pulmonary microembolism caused postoperative morbidity and mortality following major vascular surgery.

Saldeén (6) and Modig (50) have also demonstrated that patients dying from posttraumatic respiratory problems have microemboli consisting of platelets, red and white bloodcells in the microvasculature and these vessels are surrounded by interstitial edema. Although this is a later stage, it does not rule out the importance of platelets in the early postshock period.

Endotoxin shock and trauma are known to activate platelets and produce PPT (16, 18, 31, 45, 51). In sepsis there is an almost immediate response to shock induction, but following trauma this reaction is considered to be a gradual process - a rather slow bombardment of platelet aggregates in the pulmonary vasculature. However Elmér et al. (52) demonstrated that SFP of blood decreased after passage over the pulmonary circulation at an

early stage following trauma, indicating PPT earlier in the posttraumatic course than previously suggested.

Thus platelets seem to have a role in the early phase of post-shock pulmonary reaction.

Following trauma the local hemostatic mechanism starts up in the shape of platelet adhesion and aggregation in the injured vessels presumably due to the collagen in the vessel wall (53). This is followed by the release of platelet constituents (prostaglandins, ADP) which promote further aggregation. In some cases - following major trauma or sepsis - the formed platelet aggregates lose their attachment to the vessel walls and are embolized. An increased number of circulating microemboli has been shown in experimental shock models (54, 55). Probably these microaggregates are caught in the pulmonary microvasculature causing PPT, but the further release of aggregating agents and local changes in the lungs such as endothelial damage, probably enhance PPT (46, 56).

The release of ADP, serotonin, TxA etc following platelet aggregation is undisputable (57, 58)², and this causes further platelet aggregation and PPT. This is probably a prompt and immediate reaction resulting in the early PPT and explaining the direct postshock decrease in the number of circulating platelets. Mechanical blockage of platelets have been suggested to be the cause of increased pulmonary vascular resistance, PVR (44, 59)

but most investigators conclude today that PVR is increased by vasoconstriction (45, 46). ASA has been demonstrated by Rådegran et al. (47, 48) to inhibit the rise in pulmonary artery pressure following the infusion of a platelet aggregating substance, but without preventing the entrapment of platelets in the lungs. It is important to note that in their study ASA did not prevent increased pressure, which means that PPT could be partially responsible for increased PVR.

Preventive measures and drugs

The prevention and treatment of ARDS is still a matter of controversy. The benefit of early institution of fluid therapy for restoration of normal circulation, early respiratory support, early antibacterial treatment, and cardiovascular support if needed seems indisputable. Specific treatment for prevention of the development of respiratory failure is however not yet defined and is still under debate.

Platelets have a probable and early role in this pathophysiologic postshock event. Therefore it seems natural to try to inhibit the reaction of platelets early on in shock. It is known that following platelet aggregation the release of TxA₂ accelerates further aggregation. It is also known that PGI₂ produced in the vascular endothelial cells has an inhibitory effect on aggregation (60, 61). In vivo PGI₂ and TxA₂ are suggested to be

in balance which is important for the normal haemostasis. Following shock this balance is more or less disturbed causing PPT (62, 63). The prostaglandins also have effect on the pulmonary vessels, where prostacyclin dilates and IxA constricts the vessels, resulting in changes in the vascular resistance (60, 61). Drugs modulating this balance, for example PGE with a similar but less potent effect than PGI would thus theoretically be of value in the treatment of PPT.

Acetyl salicylic acid (ASA) inhibits collagen induced platelet aggregation but has also an inhibitory effect on prostaglandin synthesis. Thromboxane production is irreversibly inhibited while inhibition of prostacyclin synthesis with ASA is reversible within a few hours (60).

Ticlopidine hydrochloride (64) is a drug with an antiplatelet effect. The mechanism by which the drug acts inhibiting platelet aggregation is still undefined. However, it has been demonstrated that oral administration is necessary to achieve its effect, so the inhibitory effect on platelets is probably caused by a metabolite (65, 66). Inhibition of the release reaction has been shown (67) but no effect on prostaglandin synthesis or cyclic AMP level (65, 66).

Sorrells et al. (68) demonstrated in dogs that injection of PGE before induction of endotoxin shock reduces the increase in

pulmonary vascular pressure, and Demling et al. (69) was able to demonstrate a similar result in experiments where prostacyclin infusion was followed by endotoxin shock. PGE₁ and PGI₂ are supposed to have a similar effect, though with different potency and it is therefore possible to compare results from experiments with these two drugs.

When ASA (47, 48) or indomethacin (70) treatment is used a similar reduction in pulmonary vascular pressure has been shown. This is attributed to the effect of these drugs on cyclo-oxygenase by inhibiting TxA₂ and PGI₂ formation (61, 71).

Fletcher and Ramwell (72) showed that following endotoxin infusion there was an increase in pulmonary artery pressure but this was not altered by PGI₂-infusion. They also demonstrated a transient attenuation of thrombocytopenia and that prostacyclin alone did not significantly change pulmonary artery pressure.

In the clinical situation when the postshock reaction with PPT has already started the above mentioned types of drugs are probably less effective. Therefore other drugs directed towards steps in the further course are tried.

Some advocate the use of corticosteroids (73, 74) while others consider the drug not effective or even disadvantageous and dangerous (75). The benefit from steroids should be a reduction

of the alveolo-capillary permeability, an inactivation of the complement system and perhaps an inhibiting effect on platelet aggregation (73, 74, 76). However Blaisdell (75) opposes the use of the drug due to its immunodepressant effect making the patient more susceptible to infections. Probably high-dose short term use early on in the course could be of benefit for the patient.

Drugs with anticoagulant or antithrombotic effects e.g. heparin (41) or dextrane (20) have only resulted in a modification of the reaction, but have a doubtful effect on the end result, and sometimes even have a negative effect in trauma cases due to the bleeding risk.

Lung denervation by autotransplantation has been suggested to protect the lung from changes in pulmonary hemodynamics and pulmonary edema characteristic of early respiratory failure after hemorrhagic shock and cerebral hypoxia (34, 77, 78, 79). Moss (79) showed that pulmonary hemodynamic changes in dogs subjected to hemorrhagic shock were prevented by autotransplantation of the lung. Different brain insults including hypoxic perfusion (80) produce neurogenic pulmonary edema (NPE) supposedly mediated over sympathetic nerves (33).

Grauer et al. (34) demonstrated that increased intracranial pressure in dogs caused PPT, and surgical denervation protected the lung.

Stallone et al. (32) were not able to substantiate a similar protection. Blaisdell and Lewis (81) found interpretation of this type of experiments difficult due to the surgical procedure in the lungs. Moreover, they considered this experimental situation described by Moss, with extreme anoxic brain so abnormal that it could not be compared to a clinical situation.

The effect of alcohol

Acute alcohol intoxication is often seen in connection with severe trauma. Effects of alcohol such as depressing of CNS and respiration make these patients more prone to complications, for example respiratory failure. It is also known that alcohol affects platelet function (54), but the mechanism is unknown. However Bengmark et al. (82) have shown that the number of circulating microaggregates increased in ethanol intoxicated pigs. Elmér et al. (52) studied the effect of trauma in acute ethanol intoxicated pigs and they showed that the screen filtration pressure (SFP) increased in blood from vena cava and aorta following ethanol intake, and that trauma caused further increase in SFP in the venous blood but not in the aortic blood.

These findings and a simultaneous increase in lung capillary wedge pressure indicate platelet sequestration in the lungs.

Alcohol intoxication also has a cardiac depressant effect which in experimental animals was potentiated by exercise as demonstrated by Stratton et al. (83). Other studies have shown a peripheral vasodilator effect (84), which explains why intoxicated patients withstand blood loss poorly. On lung vessels Doekel (85) demonstrated the vasopressor effect of alcohol causing increased pulmonary vascular resistance, perhaps due to the formation and pulmonary trapping of microaggregates described by Bengmark et al. (82).

It is likely that other drugs daily used in modern society have a similar negative effect following trauma situations.

Since it is almost impossible to predict the risk for the development of postshock respiratory failure, it is important on the one hand to search for the etiologic factors which may form the basis for a specific treatment and on the other hand to search for unspecific preventive measures such as drugs.

A possible way could be to use a model for experimental platelet trapping and to evaluate the effect of shock of different etiology and an eventual result of treatment with different means.

Thus the first step is to find a suitable shock model, that produces PPT. The model should be reliable, easy to perform and be reproducible. The second step is to find a model to study PPT over a period of time in vivo pre - and postshock, which enables studies of dynamic PPT. After resolving this step the method might be modified and adapted for clinical use.

AIMS OF THE PRESENT INVESTIGATION

- I - to evaluate an endotoxin shock model and to study the effect of two antiplatelet drugs.
- II - to evaluate the possibility of a central nervous system axis, responsible for pulmonary platelet trapping following endotoxin and traumatic shock.
- III - to develop a new method enabling dynamic in vivo studies of pulmonary platelet trapping (PPT).
- IV - to study the inhibitory effect of PGE₁ and Ticlopidine on PPT.
- V - to evaluate the applicability of the dynamic method on traumatic shock.
 - to study the effect of acute alcohol intoxication on PPT.
- VI - to investigate central hemodynamics following soft tissue trauma in acute ethanol intoxicated pigs.

MATERIAL AND METHODS

Experimental animals:

Sixty-two mongrel dogs weighing 10 - 16 kg subjected to endotoxin or traumatic shock or for control experiments were used (I, II).

A total of 29 rabbits, Swedish country breed, weighing 2,0 - 2,6 kg were utilized in experiment III and IV. In investigations V and VI Swedish domestic pigs weighing 25 - 30 kg were used.

Anesthesia

Dogs and rabbits were anesthetized with pentobarbitone sodium i. v. and to maintain adequate anesthesia repeated doses of the drug were given.

In the pig experiments anesthesia was introduced with thiopenthone and maintained with fentanyl and alcuronium chloride, together with inhalation with 50 % nitrous oxide.

Drugs

Acetylsalicylic acid (ASA) - 32 mg was given orally one day prior to the experiment.

Prostaglandin E₁^R (Prostin VR UpJohn, USA and Sweden), - in dogs 50 µg/kg bodyweight 10 minutes prior to shock and a repeated

dose one hour postshock. In the rabbit experiment the same amount over 2 minutes, in association with induction of shock.

Ticlopidine hydrochloride (Ticlopidine^R, Sanofi, France),
- 150 mg/kg bodyweight orally daily, starting three days before the experiment.

Ethanol 99,5 % (Vin- och Spritcentralen, Sweden), - via a gastric tube 15 ml/kg bodyweight diluted to 40 % with normal saline.

Normal saline solution, - via a gastric tube 15 ml/kg bodyweight.

Platelet separation and labeling procedure

Dogs:

The platelet labeling was done with ⁵¹Cr according to a method described by Abrahamsen (131). From the anesthetized dog 100 ml blood was collected from the saphenous vein in a Fenwal bag (Fenwal Laboratories, Illinois) containing 30 ml ACD solution and centrifuged at 250 g for 15 minutes. The platelet rich plasma (PRP) was transferred into another Fenwal bag, and centrifuged at 1100 g for 20 minutes. The plasma was removed and saved, and the remaining platelet pellet was resuspended in 10 ml of labeling solution (Ringer solution 7 ml, citrate 60 mg, glucose 50 mg, 3 ml sterile water). To the resuspended platelets 15 MBq Na⁵¹CrO₂ 4

was added and the cell suspension gently mixed in room temperature for 45 minutes. Some of the saved plasma was added to the cell suspension followed by centrifugation at 1100 g for 20 minutes. The supernatant was removed and the remaining cell pellet resuspended in the rest of the saved plasma. Two ml of 5 % ascorbic acid was added and the cell suspension was injected i. v. into the animals. The dogs were anesthetized during the labeling procedure which lasted about two hours, during which time the animals recieved approximately 200 ml normal saline solution.

Rabbits and pigs:

The labeling procedure in rabbits and pigs was done with ¹¹¹In-oxine as described by Hardeman (86) with modifications adapted to the platelets of the respective animal.

Whole blood 15 ml and 100 ml, respectively, was collected with ACD-NIHA solution and centrifuged at 130 g for 20 minutes. The platelet-rich plasma was transferred and pH was adjusted to 6,5 with ACD-NIHA solution. The platelet-rich plasma was centrifuged at 500 g for 10 minutes and left to stand for 30 minutes. All plasma was removed and the remaining platelet button was resuspended in phosphate buffered saline. ¹¹¹In-oxine approximately 6 MBq and 20 MBq, respectively, was added and the cellsuspension was incubated for 15 minutes. The whole procedure was performed in room temperature.

50 μ l of the cell suspension was saved and the rest reinfused into the animal.

Labeling efficiency was determined by centrifugation of the 50 μ l labeled cellsuspension in a hematocrit capillary tube for 5 minutes at 700 g. The tube was then cut just above the packed platelets and the labeling percentage was determined by comparing the activity in the cell and plasma fractions measured in a NaI(Tl) well counter (Selektronik 45 - 22).

The animals were put briefly to sleep for blood sampling. The labeling procedure took approximately 2 hours and was started 24 and 8 hours, respectively, prior to the experiment.

Radioactivity measurements and scintillation camera technique:

Dogs:

After the experiments approximately 2 ml blood was taken. The lungs were removed, weighed and dried with pressurized air to constant weight. The lungs were divided and placed in tubes and the ⁵¹Cr activity was counted in a gamma scintillation counter (Nuclear Chicago Gamma Scintillation counter model 1195) together with the blood drawn prior to sacrifice.

Rabbits:

During the whole experiment the animals were put in supine position under a scintillation camera (Searle Pho-Gamma III HP, Siemens) equipped with a 4000 hole parallel hole collimator. Sequential 60 second images were taken for 35 minutes to 1 hour. The first 5 minutes were used as reference values of the normal distribution of the ^{111}In . In labeled platelets, whereafter shock was induced. Images were stored on a computer (Gamma 11, Digital Eqp). After the completion of the study, regions of interest (ROI) were selected over the heart and lungs and time activity curves generated (figure 1). From the count rate in ROI, $N(t)$, the real activity, $A(t)$, could be calculated with a calibration factor $81,9 \pm 2,6 \text{ s}^{-1} \text{ MBq}^{-1}$ obtained in a rabbit phantom for the actual geometry using the formula:

$$A(t) = N(t) / 81,9 \cdot \Delta t$$

where Δt is the measured time interval.

(1)

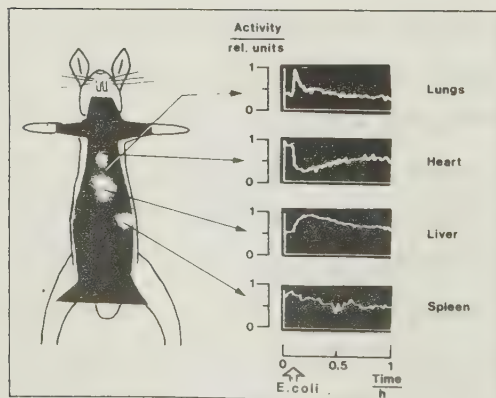


Fig. 1. The time-activity curves generated for different organs following IV injection of ^{111}In -labeled platelets. The induction of the endotoxin shock (indicated by arrow) is clearly seen in the lungs, heart, and liver curves

Pigs:

The pigs were put under the scintillation camera carefully fastened in a specially molded plastic stand to secure unchanged position during the experiment. Registration time was 140 minutes during which time sequential 60 second images were recorded. Otherwise the scintillation camera technique was similar to the rabbit model.

Pulmonary platelet trapping and wet/dry lung weight

Dogs:

The lung - blood ratio of the specific activities was determined as a measure of pulmonary platelet trapping.

As a measure of pulmonary edema the wet/dry lung weight ratio was determined.

Rabbits:

The activity curve over the lung area represents the sum of the platelets sequestered in the lungs and circulating platelets in the blood. The trapped platelets could therefore be calculated using the following formula

$$A_s(t) = A_l(t) - k \cdot A_h(t) \quad (2)$$

$A_s(t)$ = sequestered lung activity

$A_l(t)$ = sequestered lung and blood activity.

$A_h(t)$ = heart activity (proportional to blood activity)

k = lung/heart activity ratio before shock

Pigs:

The same method as in the rabbit model.

Hematologic recordings

Platelet aggregation:

Dogs:

Blood samples were drawn before and after shock and platelet rich plasma was isolated for study of ADP-induced platelet aggregation using a photoelectric method (Colysagraph Damon, IEC Division, Mass, USA).

Blood activity measurements, platelet counting and hematocrite:

Rabbits:

Blood samples were collected in EDTA tubes. One fraction (10 μ l) was taken for platelet counting using phase contrast microscopy. Another fraction was taken for determination of hematocrit and the rest for measuring ¹¹¹In activity in blood in an automatic sample-changing NaI(Tl) well counter.

Pigs:

Similar as for the rabbits but additional blood samples were taken for blood gas analysis using conventional methods on heparinized blood and temperature correction.

Hemodynamic and ventilatory mechanic recordings

Dogs:

An arterial catheter was placed in the right brachial artery for blood pressure determination.

Pigs:

The carotid artery and the internal jugular vein on the left side were cannulated. In the right jugular vein a thermodilution catheter (Swan Ganz 7 F) was introduced.

The three catheters were connected to pressure transducers (Siemens Elema EMT 34) for the recording of :

mean arterial pressure (PAo) - mm Hg

mean right atrial pressure (Pra) - mm Hg

mean pulmonary artery pressure (PAP) - mm Hg

mean pulmonary capillary wedge pressure (PCW) - mm Hg

heart rate (h.r.) - beats min⁻¹

cardiac output ($\dot{Q}$) - l/min

and for the calculation of:

Pulmonary vascular resistance:

$$PVR = \frac{PAP - PCW}{\dot{Q}}$$

Stroke volume (ml):

$$SV = \frac{\dot{Q}}{H.F.}$$

Systemic vascular resistance:

$$SVR = \frac{PAo - Pra}{\dot{Q}}$$

Stroke work right ventricle:

$$SWR = SV (PAP - Pra)$$

Stroke work left ventricle:

$$SWL = SV (PAo - PCW)$$

Using a calibrated lung mechanic computer (Siemens Elema 940), lung compliance (C_{TOT}) was determined. With a carbon dioxide recorder (Siemens Elema 930) connected to the servoventilator 900 B. Following parameters could be measured - end tidal carbon dioxide concentration ($E'TCO_2$), carbon dioxide elimination ($\dot{V}_{CO_2}$) and effective minute ventilation ($\dot{V}_E$ eff.).

Operative procedures

Dogs:

In experiment II seven dogs underwent unilateral denervation of the left lung according to Moss (87). The left atrium was transected using a wire technique, which permits uninterrupted blood flow. The left main bronchus was transected and resutured and following heparinization the left main pulmonary artery was clamped, divided and resutured. The operation was carried out approximately 2 months prior to the shock experiment. Before the experiment, when the lungs supposedly had returned to normal function (88), a lung X-ray was done to confirm bilaterally inflated lungs. Blood flow in the operated lung was confirmed by ^{99m}

Tc labeled serum albumin scan (89).

Just prior to the shock experiment a tracheostomy was performed in the animals and a split tracheal tube inserted to permit evaluation of the Hering-Breuer reflex in each lung.

Histologic and electron microscopic preparations

Dogs - In experiment II coded biopsies from each lung were taken for histopathologic and electron microscopic studies.

Experimental groups

Endotoxin model:

Shock was induced by injection of endotoxin E.coli intravenously.

Dogs - 20 mg in 10 ml saline i.v. over 5 minutes (lipopolysaccharide, E.coli 0128:B12, Difco lab, Detroit Michigan).

Rabbits - 2 mg/kg bodyweight endotoxin (lipopolysaccharide, E.coli 0111:B4, Difco lab, Detroit Michigan) i.v. in the right ear over one minute.

Trauma model:

Dogs - Soft tissue trauma consisted of 200 blows with a padded mallet to each hind limb according to Blalock's description (36).

Pigs - Soft tissue trauma consisted of 100 blows to each hind limb with a rubber mallet.

EXPERIMENTAL DESIGN

I - In the first experiment five groups of animals were studied: non shock controls, dogs in endotoxin shock, dogs in-shock and ASA-treatment, dogs in shock and PGE treatment and dogs in shock and treated with a combination of ASA and PGE¹. The shock period was 2 hours, then the animals were sacrificed. Blood-pressure, PPT and wet/dry lung weight ratios were studied.

II - In the second experiment the dogs were divided into five groups - sham controls, trauma controls, trauma in dogs with the left lung denervated, endotoxin control, endotoxin in dogs with denervated left lung. Dogs subjected to soft tissue trauma were studied for 4 hours, and endotoxin shock dogs for 2 hours.

The following parameters were studied: Blood pressure, PPT, platelet aggregability, wet/dry lung weight ratios. Histologic studies and electron microscopic evaluation of the lungs were performed.

III - All rabbits in the experiment were subjected to endotoxin shock, as every animal served as its own control. During continuous recording of ¹¹¹In-activity

blood samples were drawn through a catheter in the left ear, prior to shock, directly after and 30 minutes after shock induction. Labeling efficiency, dynamic PPT blood activity and number of blood platelets were studied. Control studies were done with ^{99m}Tc labeled red blood cells to exclude redistribution of lung blood following endotoxin shock.

IV - In this investigation four groups were studied - rabbit subjected to endotoxin shock, shocked animals treated with Ticlopidine, shocked animals treated with PGE₁ and finally not shocked animals treated with PGE₁. Following 5 - 10 minutes of steady state, shock was introduced and recording of ¹¹¹In activity lasted for 35 minutes thereafter.

Blood samples were taken from a catheter in the right femoral vein prior to shock, just after and 30 minutes postshock. Besides dynamic PPT, labeling efficiency, blood activity, platelet counting and hematocrit were studied.

V-VI - Pigs subjected to soft tissue trauma were studied. Basal values were recorded after which the animals received ethanol or normal saline via a gastric tube. Ninety minutes thereafter the animals were traumatized in each hind limb in a standardized way. Scintillation camera recordings started 90 minutes prior to trauma and con-

tinued during the whole experiment, in total 140 minutes. Hemodynamics and pulmonary mechanics, hematocrit, platelet counts and blood activity were studied at steady state, pretrauma and 3, 10 and 30 minutes after trauma.

Dynamic PPT and platelet labeling efficiency were also studied.

STATISTICAL EVALUATIONS

Values are reported as mean and standard deviation.

Statistical significance was defined by the Mann Whitney U-test, and Wilcoxon's rank sum test for differences between groups, Student's t-test and the Friedman test for within group analysis.

RESULTS

Effects of Prostaglandin, Acetylsalicylic acid and Ticlopidine on endotoxin induced PPT (I, IV)

I - No sham controls showed any significant alteration in blood pressure after 2 hours of anesthesia. Endotoxin caused a significant reduction in blood pressure (60 - 110 mm Hg). This blood pressure decrease was not prevented by any of the treatment modalities during the whole experiment.

PPT was 2 hours after shock induction significant when compared with the control group. Treatment with ASA alone or in combination with PGE did not affect pulmonary platelet sequestration. A significant reduction was however demonstrated in dogs treated with PGE¹ alone. ¹

The gross appearance of the lungs after shock showed scattered hemorrhagic areas. The wet/dry lung weight ratios showed no significant change in any of the groups.

IV - The labeling efficiency in the rabbits studied in vivo was $82 \pm 7\%$ (1 SD). Hematocrit did not change significantly in any group during the 40 minutes study. PGE¹ alone did not affect platelet count, blood activity or PPT.

Administration of endotoxin resulted in distressed respiration and in some animals watery diarrhoea with no obvious difference between the groups.

The platelet count and blood activity decreased simultaneously and promptly after shock, significantly less in the PGE₁ treated animals. These parameters were at the end of the observation time still lower than prior to shock induction, but the differences between the groups were abolished. Ticlopidine treatment did not affect either of these parameters.

"Dynamic" PPT (see experiment III), visualized on time activity curves (figure 2), showed a significant increase of the activity over the lungs and a simultaneous decrease over the heart following endotoxin injection. The pulmonary increase was significantly less prominent in the PGE₁ treated animals, but Ticlopidine did not show any effect on PPT

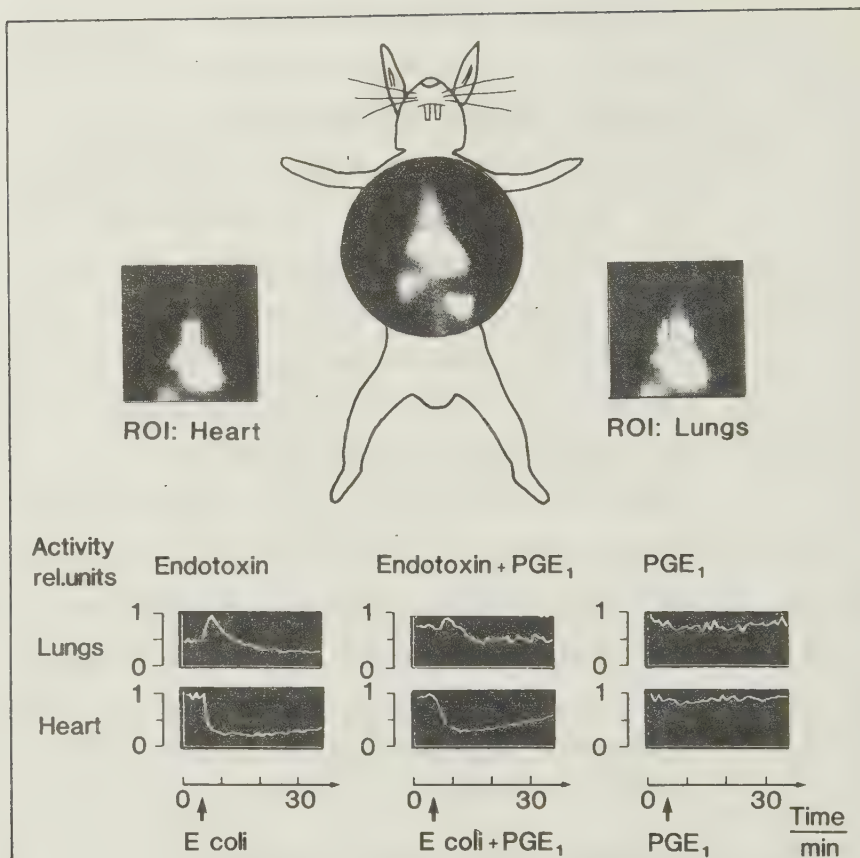


Figure 2. The scintillation camera field of view and time-activity curves generated for the heart and lung regions in three animals.

(figure 3 a). The differences are even more obvious after subtraction of the background activity (i.e. blood activity) seen on figure 3 b where Eq 2 is used. This difference between PGE₁ treated animals and the endotoxin controls is statistically significant at 3 and 10 minutes postshock.

Normalized mean activity
relative units

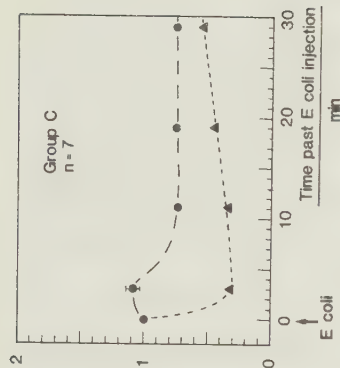
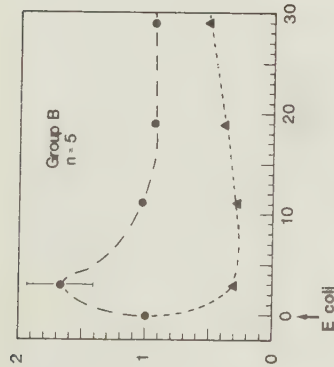
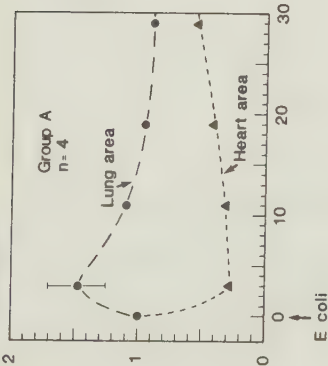


Figure 3 a. Normalized mean time-activity curves for the lungs and heart in group A, B and C. The pulmonary activity increase after shock is significantly lower in the PGF₁-treated animals, group C.

Normalized mean activity
relative units

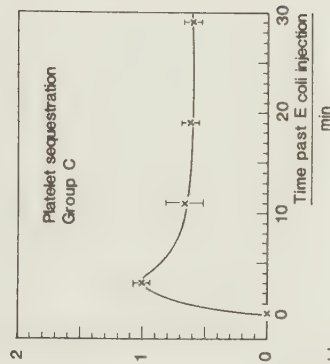
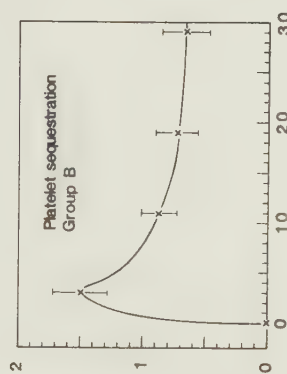
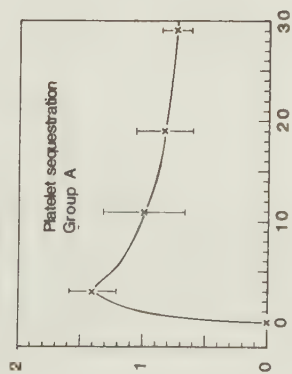


Figure 3 b. Pulmonary platelet sequestration calculated from the values in figure 3 a using equation (2). Group A - endotoxin shock, B - shock with Iclodipine treatment, C - shock with prostaglandin treatment.

"Dynamic pulmonary platelet trapping" (III)

In this experiment all animals were their own controls. The mean labeling efficiency for the whole series was $90\% \pm 5\%$ (± 1 SD). Within 2 - 4 minutes after induction of endotoxin shock the rabbits showed signs of respiratory distress. Figure 4 shows the images of one rabbit, and the change in activity-distribution following shock is obvious. From the same animal the time-activity curves generated for different organs are seen in figure 1.

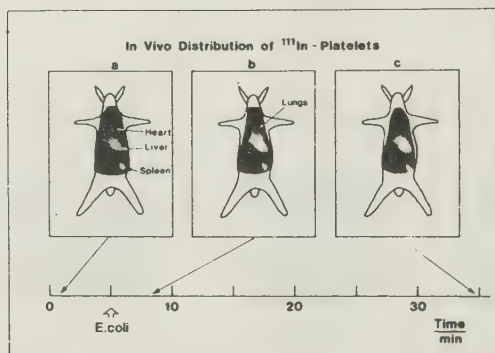


Fig 4. Images of the in vivo distribution of ^{111}In -labeled platelets, before and after E. coli injection. Note the apparent change over the lung area in Fig. 4 b compared with a and c.

"Dynamic" PPT is calculated from count rate curves with Eq 1 and Eq 2 (Figure 5 a and 5 b). As these results depend on the chosen ROI, the activity curves are normalized to unity (Figure 5 c and 5 d, respectively). These clearly show a sudden sequestration of

platelets 2 - 5 minutes after endotoxin induced shock. The following 30 minutes show a slow decrease but at the end of the experiment PPT is still significant. Platelet counts and blood activity studied in blood samples showed a simultaneous and equally large decrease following shock. This decrease corresponded well to the PPT in time after shock induction.

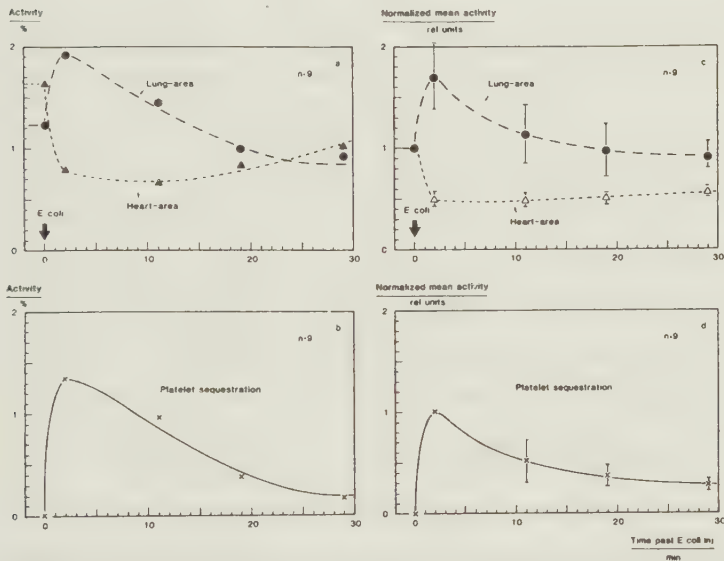


Fig 5 a-d. Time-activity curves and calculated PPT-values. a. Mean time-activity curves of percentage activity in the lungs and heart. b. Pulmonary platelet sequestration calculated from Fig 5 a and Eq. 2. c. Normalized mean activity curves for the lungs and heart. d. Platelet sequestration calculated from Fig. 5 c and Eq.2.

Effect of pulmonary denervation (II)

Sham control did not show any change in ADP-induced platelet aggregability, wet/dry lung weight ratio, blood pressure or PPT at two or four hours of observation time. Histological and electronmicroscopic studies did not show any pathology.

Endotoxin shock:

Shock induction caused a significant blood pressure fall (mean 95 mm Hg). ADP-induced platelet aggregability could not be tested after endotoxin shock because aggregation started immediately when the blood sample was taken, indicating increased aggregability. No change in wet/dry lung weight ratio was found. Significant PPT was seen in endotoxin control animals. Denervation of the left lung resulted in less pronounced PPT, a difference which was significant when compared with the normal right lung.

The histological studies showed signs of pulmonary edema, vascular congestion and alveolar collapse in both lungs of shocked animals. However in the right innervated lung pulmonary arteriolar thrombi surrounded by intra-alveolar hemorrhage were found. These were not present in the denervated lung.

Electron microscopic evaluation revealed platelet and leucocyte aggregation and endothelial damage. A significantly greater number of platelets in the normal lung than in the denervated

lung was seen. This difference between the lungs was not seen in the neutrophil counts.

Traumatic shock:

Soft tissue trauma resulted in significant blood pressure drop (mean 60 mm Hg). ADP-induced platelet aggregability was increased after soft tissue trauma, wet/dry lung weight ratio was not affected by trauma. PPT was significant following trauma but no difference between the lungs in the trauma control animals was recorded. In the unilaterally denervated dogs trauma caused a significant PPT in the innervated normal lung but no PPT in the denervated contralateral lung when compared with the sham control.

Histopathologic changes were similar to those seen in the endotoxin shock animals with the exception that in the trauma group extensive fat emboli were demonstrated. Thrombi and hemorrhage were present in the normal but not in the denervated lung.

Electronmicroscopic studies showed changes similar to those in the endotoxin dogs though to a lesser degree. As in the endotoxin animals the number of platelets in the lungs was greater in the right lung than in the denervated one. There was no difference between the lungs concerning the number of leucocytes.

The influence of ethanol on PPT (V, VI)

Pretrauma:

Labeling efficiency was $71 \pm 5\%$ (1 SD) in the saline treated group and $77 \pm 2\%$ (1SD) in the ethanol treated group.

Pretrauma recordings (90 minutes) of central hemodynamics, pulmonary mechanics, gas exchange, platelet count and blood activity were unchanged. Base excess decreased from a positive to a negative value during this period in the intoxicated animals, while animals in the saline group remained positive. This difference between the groups was statistically significant. No signs of PPT were registered during the pretrauma period.

Posttrauma:

A decrease in the mean arterial blood pressure was recorded directly after trauma and stayed low during the whole experiment, significantly lower in the ethanol treated group.

A direct posttraumatic decrease was seen regarding the platelet counts and the ¹¹¹In-blood activity, but there was no difference between the groups. Increased lung activity (PPT) followed 3 minutes after trauma, and this was significantly higher in animals pretreated with alcohol (figure 6). During the following 10 minutes a slow decrease in PPT was seen in both groups, but was still significantly higher in the alcohol group. Platelet counting and blood activity increased slowly and were almost normalized 30 minutes after trauma. The activity over the lungs

(PPT) showed a further decrease. At this time it was still higher than before trauma, but with no difference between the groups.

Normalized mean activity
rel units

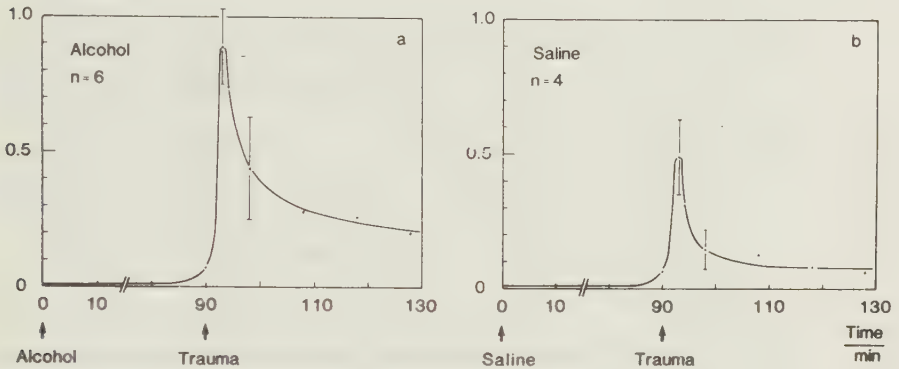


Figure 6 Pulmonary platelet trapping (PPT) $\pm$ 1SD before and after soft tissue trauma in animals pretreated with alcohol (a) or saline (b). PPT was significantly higher 3 and 10 minutes after trauma ($p < 0.02$) in animals pretreated with alcohol.

In both groups 3 minutes after trauma there was a significant decrease in blood flow ($\dot{Q}$) and it remained low during the observation period with no difference between the groups.

In the alcohol group a significant increase in PVR was recorded at 3 and 30 minutes after trauma (figure 7). No change in PVR was seen in the saline group, thus there was a significant difference between the groups during the whole posttrauma period.

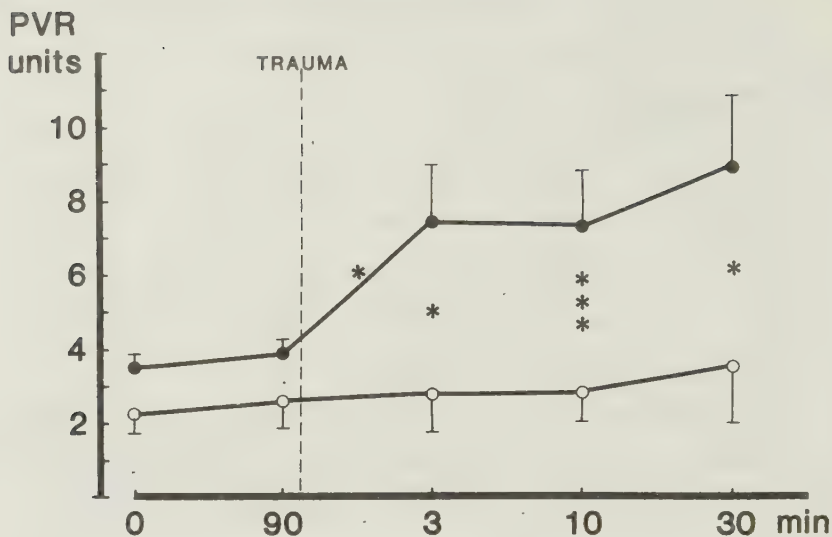


Fig 7. Mean values ($\pm$ SEM) of PVR before and 90 min after ingestion of alcohol (filled symbols) and saline (open symbols) as well as 3, 10 and 30 min after trauma.

PAP and SWR were somewhat higher during the whole experiment in the alcohol group.

Following trauma there was an increase in SVR in the two groups but without any difference, and during this period a reduction of SWL was registered in both groups.

The total lung compliance (C_{TOT}) was reduced directly following trauma, but more pronounced in the ethanol group. The following 30 minutes C_{TOT} showed a tendency to increase in the saline group while a further decrease was observed in the alcohol group.

Ten minutes after trauma there was a significant difference in PaO_2 between the groups with the higher values in the saline treated animals.

A primary reduction of E'CO_2 and PaCO_2 was seen in both groups but at the end of the observation period an increase was noted in the saline group while it was still low in the alcohol group.

$\dot{V}_{\text{CO}_2}$ was also somewhat decreased in both groups after trauma.

GENERAL DISCUSSION

Sepsis is considered to be a leading cause of the development of respiratory insufficiency, ARDS (90, 91, 92). From studies done during the Vietnam war some investigators concluded that severe pulmonary dysfunction practically never occurs without severe infection and sepsis (93, 94, 95). The validity of animal studies for application to humans may be questionable, but in many cases it is the only way to obtain information on, for example, the pathophysiology of septic shock. Lung biopsies at thoracotomy and blood samples during septic situations in humans have shown changes (96) which are reproducible in experiments done on animals (97). There has been some opinion that E.coli endotoxin injected into dogs is different from clinical gram-negative sepsis, but the present assumption is that there is little difference between all types of clinical septic shock, gram-negative or gram-positive, and that various types of experimental endotoxin shock are essentially the same (10, 98).

In our experiments (I and IV), the primary interest was PPI and the effect of three different antiplatelet drugs. A possible disadvantage in these experiments is that two different animal species were studied. There are species differences in platelet function but in an attempt to limit this difference all animals were handled in a similar way - similar anesthesia, and similar platelet labeling procedure although different labeling nuclide. Dodds (99) has shown that different animal species have different

platelet functions, but according to his results the platelets in dogs and rabbits have quite a few functional similarities. Therefore we believe that careful comparison between the results of these two experiments is permissible. In another comparative study we have shown that when our labeling method with ¹¹¹In is used, we have an acceptable labeling efficiency in both pigs and rabbits (100).

In the first experiment (I) the rabbits were kept in shock for 2 hours following endotoxin injection over a 5 minute period. The reaction with prompt blood pressure fall is well documented (16, 98) as is platelet activation and aggregation following septic shock (27, 101, 102, 103, 104). Heideman et al. (16) showed that within 2 hours the decrease in blood pressure, platelet count and PPT was consistent and at its maximum. Therefore our study was done on PPT after 2 hours.

Heideman et al. (16) and Myrvold et al. (27) also demonstrated PPT with ⁵¹Cr labeled autologous platelets by repeated lung biopsies through a thoracotomy. This procedure is questionable as the operative procedure before and during the experiment must affect the platelet function and perhaps the result of the experiment. Therefore we have chosen to remove the lung at a specific time following shock to study PPT. Probably this gives us an "exact" amount of platelet sequestered at that time, but what has happened immediately after shock is still unknown. The biopsy technique has perhaps the advantage of demonstrating the "dynamic" course of PPT. Many experiments (16, 27) have shown a

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simultaneous increase in PPT (⁵¹Cr-activity in lungs) and a decrease in blood platelet count. Thus PPT was indirectly shown using ⁵¹Cr labeled platelets.

The effect of different drugs on platelet aggregation has been studied in several in vitro experiments (57, 105). It has been shown that ADP induced aggregation causes a two phase curve, where the second phase is associated with the release of ADP, serotonin, prostaglandins etc. On the other hand other primary aggregating agents (collagen, thrombin etc) do not describe a double phase aggregation curve. It is also known that platelet aggregation can be inhibited by different drugs, for example aspirin inhibits collagen induced aggregation by inhibiting cyclo-oxygenase and thereby the production of thromboxane A₂ in platelets (57, 106, 107). PGE₁ is known to increase the concentration of cyclic AMP and is therefore one of the most powerful inhibitory agents (57, 106, 108, 109). The mentioned experiments are made in vitro, meaning that the agents inducing platelet aggregation are tested one at a time. In vivo all these agents act simultaneously and perhaps synergistically, meaning that the effect of a single specific agent is more or less important in the clinical situation. This implies that it is of value to imitate the clinical event as much as possible. Therefore in our first experiment (I) we tested two drugs, PGE₁ and ASA, in living but anesthetized animals. We have demonstrated PPT 2 hours after endotoxin shock and that ASA alone or in

combination with prostaglandin E₁ did not have any inhibitory effect on in vivo platelet aggregation using this method. However we found that PGE₁ alone significantly reduced pulmonary platelet sequestration. ASA is shown to inhibit release reaction and collagen induced platelet aggregability (106, 107, 110) and to inhibit the prostaglandin biosynthesis due to acetylation of the active side of cyclo-oxygenase, an effect which is irreversible in platelets (107, 109, 111). By contrast, the effect of ASA on, for example, prostacyclin synthesis by endothelial cells appears to be reversible within hours and also dose related - the smaller the dose the lesser the effect on prostacyclin synthesis (60, 106, 109, 112). Thus the effect of ASA on platelet biosynthesis of TxA₂ (one of the aggregating agents in platelets) is irreversible while the inhibiting effect of prostacyclin synthesis in the endothelial cells is temporary (approximately 24 hours).

PGE₁ with a similar but less potent effect than PGI₂ has an effect on platelet aggregation by its ability to increase cAMP (109), and is thus a very strong inhibiting drug (61).

In the other experiment (IV) rabbits were used but otherwise handled in a similar manner as the dogs. In this experiment we have used a new developed method (see experiment III) for dynamic in vivo studies of PPT. Pulmonary platelet sequestration following endotoxin shock was thus possible to register during the whole experiment. Endotoxin caused a prompt activity increase

over the lungs and a simultaneous decrease in blood activity and the number of circulating platelets. The change in platelet count following shock is well known from experiments (27, 98, 101, 102). Within 5 minutes there is a large decrease in the number of circulating platelets. There has been some controversy about how fast PPT occurs after endotoxin shock. However, the speed of injection and the amount of toxin injected is important and might explain the different opinion (16, 27, 113). Hasan et al. (136) made studies with endotoxin on rabbits. They concluded that the thrombocytopenia following septic shock was not affected by the injection rate but only by the given dose. However, in their "rate" - experiment they used, compared with our studies, rather large doses (5 resp 15 mg/kg bodyweight) which could be the reason for a rather fast response even though the injection speed was low. In our experiment we found that PPT was at its maximum within 5 minutes and thereafter a slow decrease but not quite "normalized" at the end of the studied period. In the two experiments (I, IV) we found an inhibiting effect of prostaglandin E on PPT. In the dynamic study the difference was significant during the first 10 minutes, and during the remaining time there was still a difference but non-significant. In the other experiment (I) there was a similar picture but with a significant difference in PPT between control animals and shock animals after 2 hours. As previously mentioned PGE has a strong antiaggregating effect (61) which we have shown in these studies. PGE has also other effects for example on the hemodynamics. It

is known to be a strong vasodilator, leading to blood pressure decrease (114) and changed pulmonary vascular resistance (115). Perhaps this is the cause of our results and not the platelet inhibiting effect.

To rule out this explanation dynamic studies were done in the same way with PGE₁ but with no shock. We found no change in the activity distribution over the lungs or heart following PGE₁ injection. This indicates that the explanation for decreased pulmonary activity after treatment of endotoxin shock with PGE₁ is not to be found in changed hemodynamics due to PGE₁. Our belief is that the cause of decreased PPT is the inhibiting effect of prostaglandin. The failing effect of ASA is probably partially due to the inhibiting effect on both TxA₂ and prostacyclin synthesis. The endotoxin model is also extremely potent and is probably simply "overwhelming" the effect of ASA.

Speaking in favour of the first explanation is that combination therapy with ASA and PGE₁ did not have any effect - indicating that this dose of aspirin inhibited the endogenous production of prostacyclin. Production and mobilisation of prostaglandin during endotoxin shock is known to be very strong (29, 116, 117) meaning that inhibition of the prostacyclin synthesis with ASA in endotoxin shock decreases the "protective" effect of prostacyclin on platelet aggregation. Thus the effect of added PGE₁ is negligible in the ASA treated animals compared with the normal effect of endogenous produced prostacyclin. In animals only

receiving PGE the little extra effect of this PGE¹ is added to the action of endogenous produced PGI¹, thus explaining the difference in the outcome of the experiments with and without ASA.

Finally in the "dynamic" experiment Ticlopidine was used in order to inhibit PPT. This drug is supposed to have an inhibitory effect on rabbit platelets 48 hours after oral administration of 150 mg/kg bodyweight daily (64, 118). Several experiments have documented an inhibitory effect of Ticlopidine but still the mechanism of the action is unclear. However different studies on the in vivo activity support the hypothesis that the effect of Ticlopidine is achieved by a metabolite, and that the effect on platelets is permanent and independent of prostaglandin synthesis and cyclic AMP levels (65, 66).

Indirect evidence suggests a permanent membrane effect, but it is still unclear if several mechanisms operate simultaneously (64, 65). Ticlopidine has been shown to inhibit ADP - induced aggregation (119) and inhibition of serotonin release from the platelets is also recorded (67). The studies referred to are done on platelet-rich plasma taken from animals or humans after Ticlopidine treatment and the platelets are tested ex vivo. Thus "one" aggregating agent is tested at a time. What happens following platelet activation in vivo?. In our study we have looked at PPT following endotoxin shock. This is one of the most potent substances for shock induction and platelet activation

and aggregation. One of the most potent inhibitory drugs - PGE₁ - was shown to have an effect on PPT in this model, but Ticlopidine failed to do so in this in vivo experiment. The reason for this is probably that endotoxin activates practically all (10) platelet aggregating agents - thus simply "overwhelming" the inhibitory effect of Ticlopidine. It is demonstrated that the inhibitory response to Ticlopidine treatment is dependent on the strength of the stimulus and has a different influence on aggregation induced by various agents (64, 118). Species differences is also an important factor to consider and Panak et al. (64) have demonstrated that rabbits needed a longer period of treatment (> 48 hours) than, for example humans before the inhibitory effect could be registered.

In these experiments (I, IV) it is obvious that the endotoxin shock model is too effective and potent for use when studying the effect of different drugs on platelet aggregation, but the model is applicable for PPT studies.

The third study was designed to develop a method for in vivo study of platelet aggregation and sequestration primarily in the lung - PPT. Earlier this pathophysiologic event following different causes of shock has been studied in animal models where platelets in most cases have been labeled with ⁵¹Cr (28, 85). After shock induction the activity in the lungs - as a measure for PPT - has been determined in lung biopsies at certain postshock intervals or simply by removing the lungs at a certain

time after shock as in our earlier experiments (I, II) (28, 120, 121). The disadvantages with these methods are obvious - using the multibiopsy procedure both in animal experiments and in the clinic. Beside the shock a thoracotomy is necessary and this together with the biopsy itself must affect platelets and the "patient" and subsequently the results. Removing the whole lung is probably better when it comes to studying PPT at a defined time after trauma, but what has happened in the meantime? Therefore our intention was to find an in vivo method to study PPT. Claes et al. (122) developed a method to detect platelet trapping in vivo in order to study renal allograft rejection. Jansson et al. (19) had a similar idea but used the model for PPT-studies. In both studies ⁵¹Cr was used for platelet labeling. This radionuclide is not optimal for in vivo scintillation camera measurements because of its relatively high photon energy and low photon yield per disintegration. Therefore we started to use ¹¹¹

In-oxine for platelet labeling. Thakur et al. (123) introduced this labeling technique in 1976 and it has proved to be suitable for platelet labeling and is favorable for in vivo measurements with scintillation cameras.

Several previously reported experiments have shown that endotoxin induced shock causes considerable sequestration of platelets in the lungs (16, 28, 92, 124, 125). Thus this shock model is safe and reproducible, factors we considered valuable in our attempt to find a suitable method for in vivo studies of PPT. In the

experiment the animals were put under a scintillation camera allowing us to measure the activity over the whole lungs continuously. In the above mentioned study, (19) only one limited area of one lung was measured during the experiment and the result was given in impulses recorded per 60 seconds. The advantage with our model was that apart from scanning both lungs simultaneously, images were taken continuously every minute.

Does the activity recorded over the lungfields and the heart correspond to the platelet distribution and platelet aggregation? Indicative for this is the decrease in blood activity and the simultaneous decrease in platelet count in blood following shock, which is registered in many other experiments (16, 27, 101, 102). Function and morphology studies done on platelets following radiolabeling have shown that the activity is bound to the platelets and that platelet aggregation probably does not "release" the radioactivity (126, 127, 128).

Thus the increase of the activity over the lungs and the simultaneous decrease over the heart within 5 minutes following endotoxin induced shock is caused by platelet removal from the circulation partly due to PPT.

Finally two control experiments were performed to rule out hemodynamic changes after endotoxin shock as a cause of changed activity distribution. In the first experiment i. v. injection of ^{111}In

$^{99\text{m}}\text{Tc}$ -red blood cells (129)

were injected i. v., in both cases prior to shock. The result showed no significant change in activity distribution following shock, thus ruling out a hemodynamic cause of our result.

Thus these experiments have shown that it is possible to study PPT in vivo by a noninvasive technique using ¹¹¹In-labeled platelets.

Endotoxin shock (I, III and IV) and traumatic shock are associated with abnormal platelet function (130). Increased platelet aggregability and the resulting PPT following shock is thought to play a significant role in the development of ARDS (20, 30, 37, 103, 124, 132). Myrvold et al. (31) and Vaage (132) have demonstrated that platelet aggregates occluding small pulmonary vessels is one of the causes of increased pulmonary vascular resistance following shock. This physical obstruction is believed to be aggravated by the release of vasoactive substances from the trapped platelets thus causing vasoconstriction (58, 117, 133). Circulating platelet microaggregates formed because of trauma or sepsis are thought by several investigators (49, 50, 113, 134, 135) to be trapped in the lungs being the first capillary bed to be encountered by the peripheral systemic platelets, thus acting as a filter. The importance of microaggregates is debated. Stallone et al. (32) demonstrated mechanical occlusion by emboli following lower limb ischemia in dogs with concomitant pulmonary edema and intra-alveolar hemorrhage but when the mechanical blockage factors were

eliminated by right lung artery anastomosis to the superior caval vein the pathophysiologic changes except for the emboli were still present. This could be explained by the release of vasoactive substances following platelet aggregation in the left lung. These substances enter the systemic circulation and finally reach the right lung causing vascular spasm and increased vascular permeability. These data do however not exclude the primary importance of microembolisation as the observation time was 24 hours, instead they could be an indication that microaggregates initiate a cascade of reactions which in the long run causes the development of respiratory problems. Stallone et al. (32) created portocaval transposition in order to make the liver the first capillary bed, and noted microemboli in the portal vein but not in the lungs, while Thomas et al. (137) showed a slight reduction in PPT but no increased hepatic trapping of platelets in a similar model. The difference is probably due to different observation time, where Stallone et al. studied the lungs 24 hours after trauma, while the other study was finished after a couple of hours. Still this does not exclude the importance of platelet aggregation in the lungs.

Thus microemboli of platelets, and/or vasoactive substances released by aggregating platelets could be responsible for the acute pulmonary damage following shock. Another cause that is discussed is the importance of neurogenic factors. Severe head injuries sometimes produce neurogenic pulmonary edema (138). This

phenomenon has been ascribed to acute systemic hypertension followed by left ventricular failure (139). Other authors do not believe in cardiogenic causes, but instead think that outpouring neural impulses from the injured brain cause systemic and pulmonary vascular constriction which in turn increases the capillary pressure causing pulmonary edema (33, 140). Sugg et al. (77) demonstrated that the pulmonary changes following hemorrhagic shock were deviated by pulmonary autotransplantation. Grauer et al. (34, 35) studied NPE following brain compression with air and platelet sequestration in the lungs. They concluded that increased intracranial pressure causes pulmonary edema and platelet activation with sequestration in the lungs and subsequent release of vasoactive substances. They also demonstrated that pulmonary denervation protects the lungs from both edema and PPT. Moss (141) postulated that shock causes hypoxemia resulting in perfusion of the brain with hypoxic blood which in turn would cause neurogenic impulses resulting in pulmonary edema. Stallone et al. (32) demonstrated pulmonary pathology following lower leg ischemia despite lung denervation. One fact remains, brain damage is sometimes associated with pulmonary edema, but the importance of neural traffic following shock is still unclear. The mentioned animal experiments are impaired by some weak points. The autotransplantation may cause changed hemodynamics on the operated lung, even if steps have been taken to rule this out, which could explain less edema and absence of platelet trapping.

Our experiment demonstrates that lung denervation is complete by the absence of the Hering-Breuer reflex (142). We also showed no change in lung circulation with pulmonary scanning (89) and pulmonary X-ray showed that the lungs were normally inflated. Sham controls (including dogs with denervated left lung) did not develop any lung pathology during anesthesia.

The difference in the observation time between the shock groups is due to the opinion that PPT occurs earlier following shock induced by endotoxin (16, 28) than soft tissue trauma (18, 21, 103, 120). No difference was found in the wet/dry lung weight ratio between groups or between right and left denervated lungs. This is explained by the fact that these experiments are designed to show pulmonary platelet trapping, which is an early manifestation before edema has developed.

In both shock groups there is significant PPT and in the trauma group this is totally deviated by lung denervation, while it is only partly deviated in the endotoxin model. Endotoxin i.v. is an extremely potent shock inducer which explains this difference between the models. There are histopathologic changes in the two shock models of similar appearance, but more pronounced in the endotoxin shocked animals. In the denervated lungs thrombi with intra-alveolar hemorrhage were missing, otherwise they were of similar appearance as in the innervated lungs. Electron microscopic studies showed platelet aggregates following shock but these were more pronounced in the normal lungs than in the denervated lungs. Neutrophil aggregation was also present but

more prominent in the endotoxin group though there was no difference between the denervated and the normal lungs.

Thus denervation has shown absence of or diminished PPT⁵¹ demonstrated with Cr-labeled platelets and platelets counted in the lungs with electron microscopy. Hemodynamic causes of the difference have as far as possible been excluded with lung scan, X-ray and examination of the vascular and bronchial anastomosis in the lungs after the experiment. The number of neutrophils counted in electron microscopy showed no difference between the normal and the denervated lung which is also indicative of no hemodynamic explanation for the difference in PPT.

Our findings support the relevance of a central neurogenic pathway in the development of early postshock pulmonary changes.

Respiratory insufficiency (ARDS) is often a critical complication in major trauma and correlates with a significant increase in mortality (5, 143, 145). The clinical manifestations usually start later than 24 hours after trauma and are dominated by tachypnoea and increased respiratory effort. Patients who die during the first hours after severe trauma show at autopsy microemboli consisting of fibrin, platelets, red and white blood cells in the pulmonary vessels (6, 50). The importance of platelets in the early posttraumatic course and in the late development of respiratory problems is debated. Here, much interest has been focused on platelets, as the mentioned

posttraumatic complication is associated with early pulmonary entrapment of platelets and microaggregates (6, 50, 146). Most experimental models where PPT following shock is studied have demonstrated gradual pulmonary sequestration hours after trauma (18, 24, 51, 120), which explains why in our previous experiment (120) performed on dogs, we removed the lungs 2 hours following trauma for PPT studies. In that study PPT was moderate but significant at that time. However, our dynamic experiment has shown that PPT is an almost immediate event following soft tissue trauma, which perhaps could support the assumption that platelet aggregation and PPT is an early etiologic factor for the development of traumatic respiratory failure. This early platelet reaction is similar to but less prominent than that seen in our experiments following endotoxin shock (III, IV). Almdahl et al. (147) demonstrated in their experiments on cats that lung embolization with coagulated autologous blood caused platelet trapping in the lungs 3 - 4 minutes after embolization, but already 10 minutes later the platelets trapped in the lungs had disappeared. The experiment shows that the embolus in itself causes platelet mobilisation and aggregation, but in the clinical situation the cause of the embolus (for example trauma, or an iliac vein thrombus) must be of importance for the further course - for example activated coagulation system, liberation of tissue thromboplastin, tissue necrosis etc. Our studies have shown that shock causes an initial PPT which after a recovery period of 30

minutes is reduced but still present. Thus we have found that dynamic studies of PPT are possible after trauma.

Alcohol is often the genesis for trauma and it is found to be present in half of the trauma patients, which Elmer and Lim (148) showed in a prospective study. This is in accordance with other similar studies (149, 150). The effect of acute alcohol intoxication on platelets has been studied by Bengmark and Elmer (52, 54, 82). They demonstrated that low blood alcohol concentration resulted in increased bleeding time and impairment of platelet aggregation in healthy volunteers. They also concluded that high blood concentration of alcohol induced formation of platelet microaggregates in the circulation in pigs. They also demonstrated that the number of microaggregates increased further after trauma in acutely intoxicated animals. Besides having a CNS-depressant effect, alcohol is suggested to have direct cardiodepressant effect, which could be responsible for the increased mortality noted in experiments done on animals (83, 151, 152). Alcohol also has a peripheral vasodilator effect and intoxicated patients are shown to withstand acute blood loss poorly and even minor trauma in these patients results in profound hypotension with bradycardia (84). Doekel et al. (85) have demonstrated that ethanol exerts vasopressor activity on the lung vessels. These effects on the platelets and the circulation could easily explain why we registered increased PPT following trauma in intoxicated animals. In experiment II we

showed a possible neural pathway causing PPT and pulmonary pathology. Alcohol, with its CNS-depressant effect, should thus be able to obviate the pulmonary effects. However, the results in this experiment (V) showing increased PPT after alcohol intake do not contradict our earlier results (experiment II) as the effect of alcohol on an expected neural pathway is not as complete as the surgical nervous disruption. Moreover the direct effect of ethanol on pulmonary vessels is probably more efficient than the CNS-depressant effect.

The hemodynamic differences between alcohol and saline treated animals following trauma, may be a result of differences in anaesthetic level. However during the long pretrauma observation period (90 minutes) there were no differences between the two groups in cardiac output and stroke work of the left heart, indicating similar anaesthetic depth.

Trauma resulted in increased PVR in the alcohol treated group which was probably an effect of alcohol (85), but this effect was not seen in the saline treated group. Greene et al. (144) found that high PVR increased the mortality rate in patients developing ARDS. In our study PVR was increased already 3 minutes after trauma, which indicates the role of PPT causing increased pulmonary vascular resistance. Pulmonary sequestration of platelets was at its maximum at this time, and PPT was found to be significantly higher in the alcohol group explaining the

increased PVR in the ethanol animals. However the direct effect of alcohol on lung vessels and platelets must be emphasized.

PPT decreased slowly the following 30 minutes posttrauma in both groups, but PVR remained elevated or even increased indicating that platelet plugging is not the only cause of the increase in PVR. The release of vasoactive substances following platelet aggregation and a later (?) leucocyte trapping are possible explanations for this further increase, but still the direct effect of alcohol must not be forgotten.

SWR was similarly reduced in both groups. The normal reaction to increased pulmonary artery pressure, seen in the alcohol group, would have been increased SWR. Zapol and Snider (42) have demonstrated this compensatory increase in SWR following elevated PVR in cases of severe ARDS. However their study was done later when ARDS had developed and their patients were not under the influence of alcohol - which is known to have cardiodepressant effect. Probably in that clinical study the patients were in a circulatory steady state in contrast to our pigs which received no immediate fluid therapy for the shock.

Finally the equally large decrease in CO_2 -elimination is probably a reflection of the similarly decreased cardiac output suggesting no large shunting of blood in the lungs. Supporting this theory is the high arterial oxygen tension (Pa O_2) in the two groups.

Thus in this experiment we have shown that trauma causes immediate PPT and that alcohol increases platelet aggregation and sequestration in the lungs. We have also shown that the pulmonary vascular resistance increases simultaneously with platelet trapping in the lungs indicating the role of platelets in the early phase of pulmonary problems. Finally we also demonstrated that PVR increases despite the decrease in PPT the following 30 minutes indicating that other factors are important in the early changes in the pulmonary hemodynamics.

To sum up, PPT follows endotoxin and traumatic shock. In the endotoxin situation there is an immediate PPT, increased pulmonary vascular resistance due to platelet plugging and the release of vasoactive substances and a simultaneous thrombocytopenia. In the soft tissue trauma model a similar reaction is observed with immediate PPT and thrombocytopenia but this is less dramatic than in the endotoxin model. Pulmonary vascular resistance is in this model unchanged, but if the effect of trauma is amplified by alcohol intoxication there is an increased PVR that remains despite the decreasing PPT. Clinical manifestations of ARDS usually start a couple of days following trauma, perhaps because the syndrome develops due to complications in the shock situation (for example infection, tissue necroses and toxins). This is perhaps borne out by our experiment with alcohol amplifying the trauma effect.

It is this author's belief that early PPT is of great importance in the later development of ARDS, but other factors such as leucocytes, vasoactive substances, complement activity may be as important in the complex interaction leading to postshock pulmonary insufficiency.

CONCLUSIONS

Endotoxin shock is a very potent model for platelet activation and sequestration in the lungs (PPT). Using radiolabeled platelets (⁵¹Cr) PPT is easily studied indirectly by measuring the radioactivity in lung tissue following shock. PGE₁ has been shown to significantly reduce platelet sequestration in the lungs but did in this model not show any effect on the blood pressure fall.

Aspirin alone or in combination with PGE₁ failed to affect PPT. Surgical denervation of one lung inhibited PPT following endotoxin shock and prevented it subsequent soft tissue trauma, indicating a central nervous system axis being at least partly responsible for platelet trapping in the lungs. This effect was evident not only indirectly measured with ⁵¹Cr-activity but also by counting platelets trapped in the lungs with electron microscopy.

Dynamic studies of pulmonary platelet trapping following endotoxin shock can be carried out after platelet labeling with ¹¹¹In-oxine.

PGE₁ was found to have an inhibiting effect on PPT following endotoxin shock, using the dynamic method.

Ticlopidine failed to obviate or affect PPT using the model.

Soft tissue trauma, a rather moderate model for producing PPT, was also demonstrated in this experimental method to cause platelet trapping in the lungs. Platelets were sequestered promptly already within 5 minutes following trauma after which a slow reduction of PPT followed.

Ethanol intoxication amplified the effect of trauma by significantly increasing the trapped platelets during the first 5 minutes followed by a slow decrease as in the non-intoxicated animals.

Alcohol was also shown to increase PVR at the same time as the increase in PPT. However PVR remained elevated despite a reduced number of trapped platelets - indicating that other causes than PPT and mechanical plugging (for example vasoactive substances, CNS-axis etc) are responsible for the change in pulmonary hemodynamics.

FUTURE ASPECTS

The importance of platelets in the later development of ARDS is still controversial. Probably many factors interact from the beginning when shock is induced and probably several etiologic factors are able to initiate a reaction that in certain cases finally causes ARDS. It is important to remember that ARDS is a respiratory problem that develops over some time, usually a couple of days, and that the present experiments were designed to study platelet trapping in the lungs in the immediate postshock period.

ARDS is probably initiated very early on, but sometimes the reaction is inhibited by natural means, and therefore not followed by lung insufficiency. The problem is thus not only to get an early diagnosis, but also to find why some patients, despite being badly injured or septic, do not develop signs of respiratory distress. Perhaps there are steps in the reaction that are more essential than others so that there are some weak points in a chain reaction. Finding these would be of great help but this almost implies knowledge of the whole reaction system. In the search for this every possible factor has to be studied, probably especially those found to react early after shock.

Platelets are known to cause PPT very early following endotoxin shock and we showed that trauma also causes an early trapping of platelets in the lungs. We also found that alcohol caused

increased PPT and a simultaneous increase in the lung vascular resistance directly following trauma. This makes platelets interesting and the suspicion that platelets have an important role in the early postshock respiratory problems is enhanced. The dynamic model used in the present study may open possibilities for further studies on animals in order to survey different ways to obviate PPT by using antiaggregating drugs and drugs with effects on humoral factors (serotonin, prostaglandin, histamin) and CNS.

It is difficult and sometimes incorrect to transfer results in animal experiments to man, mainly due to species differences. The clinical situation is also difficult to mimic but our dynamic method with ¹¹¹In-labeled platelets enables experiments that more closely resemble a clinical situation. Besides animal experiments, it is also possible to use ¹¹¹In-labeled platelets in patients, but in a clinical situation the labeling procedure must be done after trauma, thus missing the acute PPT. There is perhaps in patients developing ARDS "a second wave" of platelet trapping in the lungs - indicative of this are studies done by among others Saldeén et al. on autopsy material from patients who have died from clinical ARDS. They found microemboli of platelets in the lungs. Sibbald et al. have made clinical investigations with ¹¹¹In-labeled leucocytes in patients with clinical signs of ARDS. They found increased lung activity in these patients. Perhaps this supports the opinion that ARDS

develops due to posttraumatic complications with an infection - thus they just perform an "abscess" scan with labeled leucocytes.

My opinion remains that platelets are important to study - and in the clinical situation, it may be possible to use ¹¹¹In-labeled platelets in patients following traumatic shock or major surgery (for example vascular surgery) in order to get an early indication that lung problems are under way, thus enabling early institution of treatment.

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EFFECTS OF ACETYSALICYLIC ACID AND PROSTAGLANDIN ON ENDOTOXIN-INDUCED PULMONARY PLATELET SEQUESTRATION

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Abstract. Previous experimental studies have shown that soft tissue trauma and endotoxin shock cause pulmonary platelet trapping. In the soft tissue trauma model it has been found that this event can be obviated by pretreatment with aspirin and prostaglandin E_1 in combination. This study was made to determine the effect of these two drugs on endotoxin-induced pulmonary platelet trapping. Dogs with ^{51}Cr -tagged platelets were injected with 20 mg endotoxin *E. coli*. The dogs were kept in shock for 2 hours and then sacrificed. The amount of platelet sequestration in the lungs was determined by measuring the ^{51}Cr activity in the lungs. The results of the study showed that prostaglandin alone decreases pulmonary platelet trapping induced by endotoxin, but aspirin, either alone or together with prostaglandin E_1 , has no positive effect. This could be explained by the fact that acetylsalicylic acid depresses endogenous prostaglandin, and thus decreases the blood concentration of available prostaglandin.

Soft tissue trauma and endotoxin-induced shock are associated with pulmonary platelet trapping (PPT) in dogs (Ljungqvist et al., 1971; Heideman et al., 1979). In the soft tissue trauma model, acetylsalicylic acid (ASA) and prostaglandin (PGE_1) both decreased the in vitro aggregability of the animals' platelets, but neither drug alone reduced the incidence of pulmonary platelet sequestration. However, a combination of PGE_1 and ASA did effect significant reduction in PPT (Thörne et al., 1979). The aim of the present study was to determine the effects of ASA and PGE_1 alone and in combination, on PPT associated with endotoxin shock, and to determine if these effects are similar to those seen with the soft tissue trauma model.

MATERIALS AND METHODS

Twenty-eight mongrel dogs of both sexes weighing 10-16 kg were anesthetized with pentobarbital sodium. Autologous platelets were labelled with radioactive chromium (^{51}Cr) according to the method of Abrahamsen (Abrahamsen, 1968) and reinfused into the animal. The

following day the dogs were reanesthetized and endotracheally intubated but received no ventilatory assistance. Blood pressure was followed during the shock period and approximately 100 ml normal saline was administered during this time. Twenty mg of endotoxin (*E. coli* 0128:B12, Difco Laboratory, Detroit, Michigan) was administered i.v. over a five-minute period. Two hours later blood was drawn for measurement of ^{51}Cr activity and the dogs were sacrificed. The lungs were removed, weighed and dried to constant weight with pressurized air. The dried lungs were then divided, placed into tubes and the ^{51}Cr activity counted in a gamma scintillation counter (Nuclear Chicago Gamma Scintillation Counter Model 1195) together with blood drawn prior to sacrifice. The ratio of counts/gram of lung/blood was determined as well as the wet/dry lung weight ratio.

The dogs were divided into five groups: (1) non-shock controls, sacrificed after two hours of anesthesia (7 dogs); (2) dogs with endotoxin-induced shock receiving no medication (7 dogs); (3) dogs with endotoxin-induced shock receiving ASA 32 mg p.o. one day prior to injection of endotoxin (5 dogs); (4) dogs with endotoxin-induced shock receiving 50 mg/kg body weight PGE_1 (provided by the Upjohn Company) i.v. 10 min prior to endotoxin injection and a repeated dose i.m. one hour later (4 dogs); (5) animals with endotoxin-induced shock treated with a combination of ASA and PGE_1 in the above mentioned doses (5 dogs).

RESULTS

The animals studied during two hours of anesthesia showed no alteration in blood pressure levels. Injection with endotoxin caused a significant reduction in mean blood pressure ranging between 60-110 mmHg. Only one animal died during the study, approximately 15 min prior to the time of sacrifice.

Lungs from the dogs receiving endotoxin demonstrated scattered hemorrhagic areas which were readily visible. However, the wet/dry lung weight ratios of these lungs showed no increase (Table I). The mean lung/blood ^{51}Cr ratio in the control dogs

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Table 1

Groups	Wet/ dry lung wt.	⁵¹ Cr Lung/blood	P-value ^a	No. of dogs
2 h non-shock	4.6±0.3	0.84±0.15	<i>P</i> <0.0005	7
2 h endotoxin shock	4.2±0.4	1.79±0.49	—	7
2 h endotoxin shock + ASA	4.3±0.2	1.49±0.39	N.S.	5
2 h endotoxin shock + PGE ₁	4.9±0.6	1.00±0.23	<i>P</i> <0.01	4
2 h endotoxin shock + ASA and PGE ₁	4.6±0.7	1.66±0.49	N.S.	5

^a *P*-value for all groups compared with 2 h endotoxin shock control using Student's *t*-test.

was 0.84. The endotoxin-shocked animals receiving no medication had a significant increase in ⁵¹Cr activity in the lung with a mean of 1.79 (*p*<0.0005). In the animals treated with ASA the mean ⁵¹Cr lung/blood activity was 1.49, which was significantly greater than the control and not significantly different from the value for untreated endotoxin shocked animals. By contrast animals receiving PGE₁ had a mean lung/blood ⁵¹Cr ratio of 1.0, which represents a significantly lesser increase in PPT when compared to animals receiving endotoxin with no medication or endotoxin and ASA. In the final group treated with a combination of ASA and PGE₁, the mean ⁵¹Cr lung/blood ratio was 1.66, a significant increase over the control animals, but not significantly different from that of untreated endotoxin-shocked animals.

DISCUSSION

Endotoxin represents one of the most potent methods of increasing intravascular platelet aggregability. A marked pulmonary platelet sequestration occurs within two hours after intravenous endotoxin injection (Heideman et al., 1979). These effects are accompanied by an increase in plasma PGE₁ concentration (Anderson et al., 1972).

The effects of ASA alone, on pulmonary platelet sequestration, are similar to those noted in the soft tissue trauma model in that no significant reduction occurs. A significant disparity between the endotoxin model and the trauma model exists, however, in the other two treated groups; in the trauma model PGE₁ alone had no significant effect, while this drug did significantly reduce PPT after endotoxin shock. By contrast, combined ASA and PGE₁ treatment significantly obviated PPT in the soft tissue trauma model while in the animals with endotoxin-induced PPT the therapeutic effect of

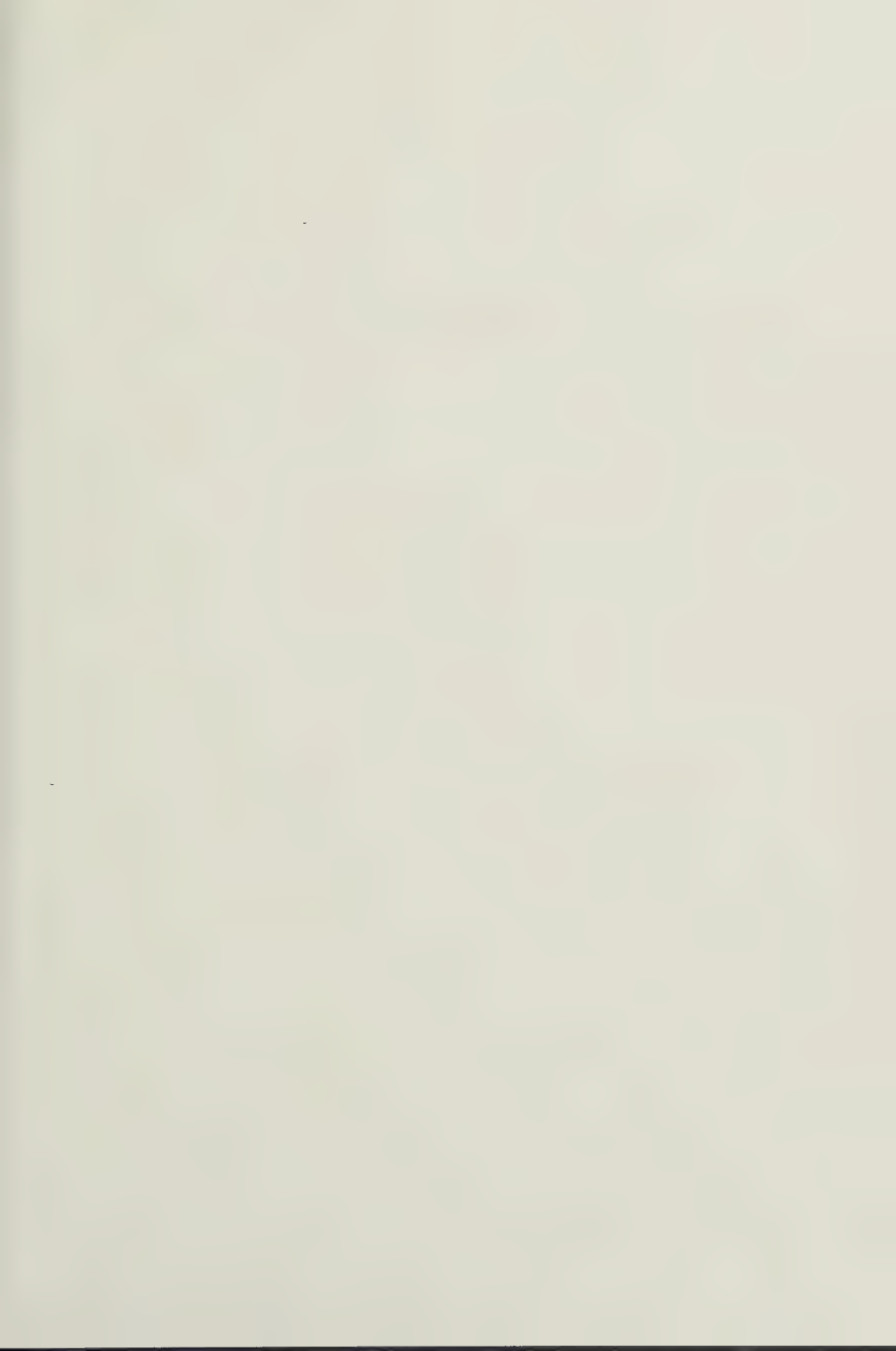
PGE₁ was counteracted by the addition of ASA. While ASA is also known to have an inhibiting effect on platelet aggregability (Weiss et al., 1968), Fletcher has shown that ASA decreases the synthesis or release of prostaglandins (Fletcher et al., 1976). It appears that the platelet aggregability reducing effects of exogenous PGE₁ and ASA combine to have a positive effect on PPT in the soft tissue trauma model. However, in the much more potent endotoxin-induced shock model, in which there is an increase of endogenous PGE₁, the predominant effect of ASA appears to be its action in reducing plasma PGE₁.

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Effect of denervation of a lung on pulmonary platelet trapping associated with traumatic shock

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We have shown previously that lung denervation reduces pulmonary platelet trapping (PPT) associated with neurogenic pulmonary edema. This study investigates the universality of the role of pulmonary innervation in PPT associated with shock induced by soft tissue trauma and by endotoxin injection. The left lungs of dogs were denervated by autotransplantation 2 months prior to induction of shock. Autologous platelets were labeled with ^{51}Cr and reinfused the day prior to shock induced either by blunt trauma to the hind limbs or by injection of endotoxin. The animals were killed after 2 to 4 hours of shock. Coded specimens of each lung were evaluated by light and electron microscopy. The remaining part of the lung was dried and counted for ^{51}Cr activity as a measure of PPT. Increased platelet aggregability was noted in all shocked animals. Hypotension induced by trauma was associated with increased PPT in the innervated lungs compared with the sham controls. There was no significant difference in PPT between the denervated lungs of these animals and the sham controls. The increase in PPT in innervated lungs was greater in the endotoxin model. Although a small increase in PPT was demonstrated in the denervated lungs of endotoxin shocked animals, this was appreciably less than noted in the contralateral innervated lungs. These findings were substantiated by light microscopy, which showed a lesser degree of intravascular thrombosis, and by electron microscopy, which identified fewer platelets and less aggregation in the denervated lungs compared to the contralateral innervated lungs. These findings demonstrate that local neurogenic stimuli affect PPT associated with shock due to a variety of causes. Drugs which reduce nerve traffic to the lung may be indicated as therapy.

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SHOCK is associated with abnormal platelet function. Chemical moieties including adenosine diphosphate (ADP), adenosine triphosphate (ATP), catecholamines, PGE_1 , PGE_2 , calcium $^{++}$, and a variety of intrinsic platelet factors released during severe hypotension cause increased platelet aggregability and adhesiveness.²⁴ Reversible aggregation and temporary trapping of platelets in various organs have been indicted as causes of thrombocytopenia in shock and trauma. Pulmonary platelet trapping (PPT) is

thought to play a significant role in the acute respiratory distress syndrome (ARDS) associated with shock caused by trauma, hemorrhage, and endotoxin.^{4, 15, 22} Myrvold and Svalander²¹ have shown that platelets are required to effect the increase in pulmonary artery pressure associated with septic shock. It has been suggested that, during shock, platelet aggregates occlude small pulmonary vessels causing increased peripheral vascular resistance.² In addition to the physical obstruction caused by platelet plugs, vasoactive substances released from these platelets may contribute to pulmonary vasoconstriction.^{24, 25}

The importance of the lung itself as a contributing factor in platelet pathophysiology also has been studied. Some investigators have proposed that the lung functions as a filter since it is the first capillary bed to be encountered by the peripheral systemic platelets.³ However, there are conflicting results from

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Table I. Comparison of lung/blood ^{51}Cr ratios

Group	No. of dogs	Left	Right	P value vs. right	P value left vs. group I left	P value right vs. group I right
I. Sham	8	$0.83 \pm 0.18^*$	0.85 ± 0.13	NS	—	—
II. Trauma (control)	10	1.17 ± 0.33	1.23 ± 0.32	NS	<0.01	<0.005
III. Trauma (denervated left lung)	5	0.83 ± 0.13	1.04 ± 0.21	<0.05	NS	<0.05
IV. Endotoxin (control)	7	1.81 ± 0.46	1.77 ± 0.53	NS	<0.0005	<0.0005
V. Endotoxin (denervated left lung)	4	1.12 ± 0.14	1.38 ± 0.18	<0.05	<0.01	<0.0005

*Mean $\pm$ SD

studies of shock caused by soft tissue trauma in which the liver was made the first capillary filter by means of a portacaval transposition. One group has noted microemboli in the portal vein,²¹ whereas our laboratory has shown no increase in hepatic platelet trapping with only a slight reduction in PPT.³⁰ Arterial platelets in rats have been shown to be less responsive than venous platelets to ADP-induced aggregation, suggesting that the lung itself has an effect on circulating platelets.⁶

The pulmonary hemodynamic effects of hemorrhagic shock in dogs have been prevented by lung denervation achieved by removal and reimplantation of this organ.²⁶ Pulmonary autotransplantation also prevents some of the pathologic consequences of shock.²⁰ Studies of the microcirculation of denervated lungs show less diminution of capillary circulation compared with control lungs, following shock.¹⁰ The present study was carried out to determine if the neural pathways to the lung play a role in the PPT that occurs in shock induced by trauma or by the injection of endotoxin.

MATERIAL AND METHODS

A total of 34 adult mongrel dogs of both sexes weighing 10 to 14 kg was divided into five groups. Group I had eight dogs, including two with unilateral denervation of the left lung were studied as sham controls. In group II 10 animals were studied as soft tissue trauma controls. Five animals with denervated left lungs were subjected to similar trauma in group III. In group IV seven dogs were used for endotoxin shock controls. Group V consisted of four dogs with denervated left lungs subjected to the same degree of endotoxin shock.

Eleven dogs underwent denervation of the left lung, according to the method of Kottmeier et al.,⁹ as

Table II. Lung/blood ^{51}Cr ratios:
Group III, trauma

Dog No	Left	Right
1	0.79	0.87
2	0.82	0.91
3	0.79	0.97
4	0.72	1.07
5	1.05	1.39
Mean	0.83 ± 0.13	1.04 ± 0.21
P	<0.05	

modified by Moss.¹⁶ The left atrium was transected using a wire technique that permits uninterrupted blood flow. The left main bronchus was divided and resutured and, following heparinization, the left main pulmonary artery was clamped, divided, and resutured. Denervation was carried out at least 2 months prior to the shock experiment. At the end of this recovery period, when the lungs returned to normal function,¹¹ the animals were x-rayed to confirm the presence of bilaterally inflated lungs. Blood flow to the denervated lung was confirmed by a $^{99\text{m}}\text{Tc}$ -tagged serum albumin scan.²³ Blood flow through the denervated lung was found to be only slightly reduced, consisting of 32% of total pulmonary flow in group III, and 35% in group V, compared with the normal left lung value of 40%.

One day prior to the shock study, 100 ml of blood was drawn from each dog, the platelets tagged with ^{51}Cr according to the method of Abrahamsen,¹ and the autologous platelets reinfused. On the following day the animals were reanesthetized. A catheter was placed in the brachial artery to permit blood pressure determinations throughout the procedure. Blood was drawn for determination of the ^{51}Cr count and for assessment of ADP-induced platelet aggrega-

Table III. Lung/blood ^{51}Cr ratios: Group V, endotoxin

Dog No.	Left	Right
1	1.28	1.42
2	0.93	1.18
3	1.14	1.60
4	1.14	1.27
Mean $\pm$ SD	1.12 $\pm$ 0.14	1.38 $\pm$ 0.18
P	<0.05	

bility, using a photoelectric technique.* A tracheostomy was performed, and a split tracheal tube inserted to permit evaluation of the Hering-Breuer reflex in each lung.¹²⁻¹⁴ All animals with unilateral denervation of the left lung demonstrated absence of the Hering-Breuer reflex in that lung, and its presence was confirmed in the contralateral lung.

Trauma imposed on group II and group III animals consisted of 200 blows with a padded mallet to each hind limb, according to Blalock's protocol.³ The average decrease in mean arterial blood pressure subsequent to trauma was 60 mm Hg. The dogs in groups IV and V were injected intravenously with 20 mg of endotoxin† over a 5-minute period. In these dogs the mean reduction in arterial pressure was 95 mm Hg. In the nontraumatized controls, group I, pressure varied only by 10 mm Hg during 2 to 4 hours of anesthesia alone.

None of the animals required ventilatory assistance during the shock period, and none died before being killed. Soft tissue trauma dogs were studied for 4 hours and endotoxin shock dogs for 2 hours, the time at which measurable endotoxin-induced pulmonary platelet trapping occurs.⁶ At the end of the shock period, blood was taken for determination of ^{51}Cr counts and for platelet aggregability studies. The dog was connected to a ventilator and the chest was opened. Coded biopsies were taken from similar sites in the upper part of the lower lobes of each lung for histopathologic and electron microscopic (EM) studies. Three separate EM grids were chosen from each tissue sample, and the intravascular platelets and neutrophils were counted in five random grid spaces of each grid. The animal was killed, the lungs removed, weighed, and dried with pressurized air for 4 to 6 days to constant weight. Wet/dry lung weight ratios were determined and the lungs were divided and placed into tubes to permit counting of ^{51}Cr

activity in a gamma scintillation counter.* Blood ^{51}Cr activity was measured at the same time and lung/blood ^{51}Cr ratios were calculated. These ratios were used as an indication of PPT. Statistical significance was defined by Student's *t* test.

RESULTS

Qualitative platelet aggregability studies showed increased ADP-induced aggregability in the two soft tissue trauma groups, II and III. Groups IV and V, subjected to endotoxin shock, evidenced increased platelet aggregability prior to the addition of ADP. The nonshock group, I, showed no change in aggregability during the 4-hour period of anesthesia.

There was no statistically significant difference in the wet/dry lung weight ratios between groups, nor was there any difference in wet/dry lung weight ratios between the denervated and innervated lungs within any group.

There was no statistically significant difference between the right and left lung ^{51}Cr measurements of the sham animals, the traumatized control animals, and the endotoxin control animals. Both lungs in the trauma control animals had a significant increase in ^{51}Cr activity when compared to the lungs of the sham animals (Table I). The mean left lung/blood ratio of the animals subjected to soft tissue trauma (group II), was 1.17 compared with a mean left lung/blood ratio of 0.83 for the sham animals. The right lung/blood mean was 1.23 compared with 0.85 in the sham animals. In the traumatized animals with unilateral denervation (group III), there was an increase to 1.04 in the ^{51}Cr lung/blood ratio in the innervated lung compared to the sham control value of 0.85, whereas there was no increase in the denervated lung (Tables I and II). There was a significant difference between the denervated and the contralateral innervated lung ($P < 0.05$).

In the animals subjected to endotoxin shock there was a marked difference ($P < 0.0005$) between the endotoxin control lungs and the sham lungs. In endotoxin shocked animals with left lung denervation there was a slight but significant increase in ^{51}Cr activity in the denervated lung compared to the same lung in the sham animal ($P < 0.01$). A more marked increase occurred in the innervated right lungs, from 0.85 to 1.38 ($P < 0.0005$), (Table I). A statistically significant difference was noted between the innervated and the contralateral denervated lungs of animals subjected to endotoxin shock (Table III).

*Nuclear Chicago Gamma Scintillation Counter Model 1195.

*Colysagraph Damon/IEC Division, Needham Heights, Mass.
†E. coli 0128-B12, Difco Laboratory, Detroit, Mich.

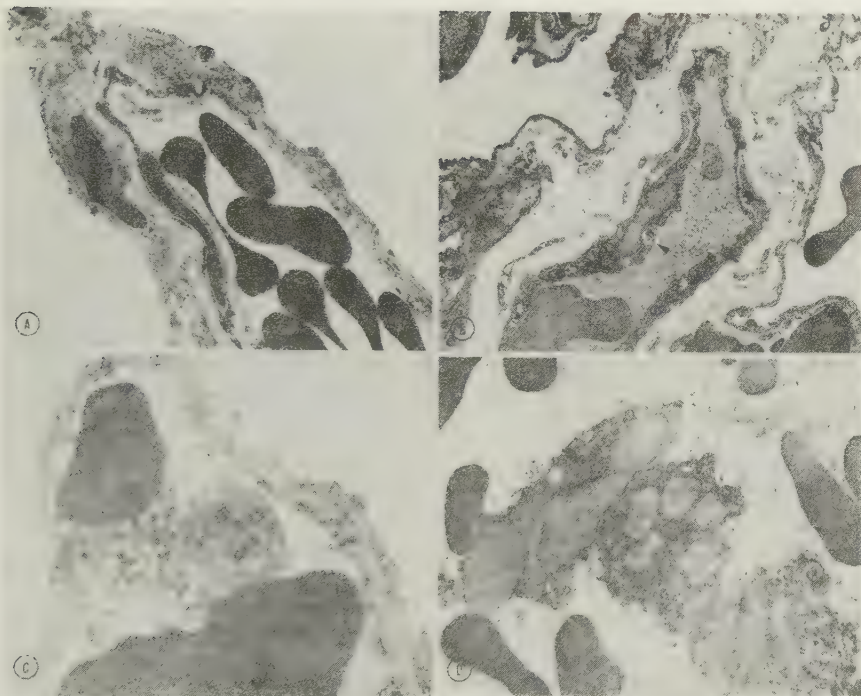


Fig. 1. A, Electron micrograph from the lung of a normal control dog showing a portion of a pulmonary venule, which contains several red blood cells. The endothelial cells are flat and the perivascular connective tissue is free of edema (Original $\times 8,400$.) B, Electron micrograph from a trauma control lung showing a portion of a pulmonary venule. The endothelial cells are swollen and demonstrate cytoplasmic degeneration in forms of lamellar body formation (arrow). The perivascular connective tissue is markedly edematous. (Original $\times 8,400$.) C, Electron micrograph from an innervated lung after endotoxin-induced pulmonary edema showing a portion of a pulmonary capillary. Three partially degenerated platelets and two red blood cells are aggregated in the vascular lumen. (Original $\times 15,600$.) D, Electron micrograph from the same tissue sample as C demonstrating intravascular fibrin deposition, a partially degenerated neutrophil, a histiocyte, and several red blood cells. (Original $\times 8,400$.)

The gross appearance of lungs from dogs subjected to soft tissue trauma was fairly normal, with a few hemorrhagic areas. The histological study showed pulmonary edema, vascular congestion, and alveolar collapse in both lungs of the shocked animals. Extensive fat emboli were demonstrated in the soft tissue trauma group. The right or innervated lungs of all shocked dogs contained large pulmonary arteriolar thrombi surrounded by intraalveolar

hemorrhage. These thrombi were not present in the denervated lungs.

Electron microscopic evaluation of the lungs of animals subjected to soft tissue trauma (Fig. 1, B) demonstrated platelet aggregation which was not present in control lungs (Fig. 1, A). The intrapulmonary platelet aggregation was more marked in the innervated lungs compared to the denervated lungs. The number of platelets counted in 15 grid spaces

Table IV. Electron microscopic platelet count in fifteen grid spaces: Group III, trauma

Dog No.	Left lung	Right lung
1	2.0	7.3
2	11.6	18.6
4	6.6	9.0
Mean	6.7	11.6

Table V. Electron microscopic platelet count in fifteen grid spaces: Group V, endotoxin

Dog No.	Left lung	Right lung
1	6.0	15.0
2	7.6	16.3
3	11.3	16.3
Mean	8.3	15.9
P	<0.005	

was greater in the innervated lung (Table IV). A greater degree of damage was noted in the endotoxin shock lung (Fig. 1, C and D). Neutrophil aggregation was more prominent in the lungs of these animals. Degranulated neutrophils within the vascular lumen were also more marked. However, no significant difference was seen between right and left neutrophil counts in either shock model. The number of platelets counted in 15 grid spaces was significantly greater in the innervated than in the denervated lungs in the endotoxin model ($P < 0.005$) (Table V).

DISCUSSION

The acute respiratory distress syndrome is a serious consequence of many types of shock. Early in the course of hemorrhagic shock, there is a transient increase in platelet adhesiveness determined by the platelet retention test and in ADP-induced platelet aggregability.¹⁴ This is associated with a decrease in the plasma level of ⁵¹Cr-labeled platelets.¹¹ Measurement of ⁵¹Cr activity in the lungs, 24 hours after the injection of autologous labeled platelets, is a reproducible method of determining PPT.^{12, 13} This is confirmed by the electron microscopic analysis of the lung specimens in this series. Both of these techniques show increased PPT following shock induced by either soft tissue trauma or by the injection of endotoxin.

The development of neurogenic pulmonary edema (NPE) follows a variety of central nervous system (CNS) injuries.¹⁷ The importance of a neural path-

way to the lung is a logical consideration in the evolution of NPE. This is substantiated by the fact that lung denervation prevents the hemodynamic changes that occur in the control, innervated lung. Recent studies from our laboratory show a reduction in PPT in denervated lungs of dogs subjected to increased intracranial pressure.⁷

The demonstration of a central neurogenic etiology of the respiratory distress syndrome in animals with hemorrhagic shock suggests that intact neural traffic may also be important in the development of ARDS associated with a variety of causes of shock.^{26, 27} Our findings support the relevance of a central neurogenic effect to the formation of ARDS in shock induced by soft tissue trauma and by endotoxin injection. Added support for this theory is offered by reports on the efficacy of drugs that interrupt the neural pathways. CNS depressants, including alpha- and beta-blocking agents have been shown to protect against NPE.^{16, 19} Further studies are indicated to determine the effectiveness of CNS depressants as therapy for the pulmonary consequences of soft tissue and endotoxin shock.

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An experimental method for in vivo studies of pulmonary platelet sequestration *

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An experimental method for in vivo studies of pulmonary platelet sequestration *

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Abstract. It has been reported in earlier in vitro studies that soft tissue trauma and endotoxin-induced shock causes pulmonary platelet trapping (PPT). This paper describes a noninvasive in vivo technique for dynamic studies of PPT in rabbits. Autologous platelets were labeled with ¹¹¹In and reinfused into the animals. The following day, the rabbits were anesthetized and placed in a supine position under a scintillation camera. Continuous measurement of the activity distribution in the animal was performed for 35 min. The first 5 min represented a preshock measurement, whereafter endotoxin *E. coli* was injected IV. The following 2-3 min showed a sudden increase of radioactivity in the lungs indicating PPT, and thereafter a slow decrease to almost the preshock level. A simultaneous decrease in the number of platelets and the radioactivity in peripheral blood also indicated the induction of PPT. This study clearly shows that PPT can be detected in vivo with an easy, noninvasive scintillation camera method.

Previous reported studies in animals with in vitro experiments have shown that traumatic and endotoxin-induced shock cause sequestration of platelets in the lungs (Blaisdell and Lewis 1977; Heideman et al. 1979; Ljungqvist et al. 1971; Myrvold et al. 1977; Thörne et al. 1979, 1981). This is thought to play a significant role in lung complications following major illness and injury. The increased platelet aggregability and adhesiveness is thought to lead to platelet entrapment by the lungs and release of vasoactive substances from the platelets causing an increased pulmonary vascular resistance (Kux et al. 1972).

In these earlier experiments ⁵¹Cr was used for platelet labeling. This radionuclide is, however, not suitable for in vivo scintillation camera measurements because of its high photon energy and low photon yield per disintegration.

In 1976, however, ¹¹¹In-oxine for cell labeling was introduced by Thakur et al. This radiopharmaceutical has proved to be suitable for labeling platelets with high efficiency. ¹¹¹In is also favorable for in vivo measurements with scintillation cameras.

The aim of the present study was to develop a noninvasive technique for dynamic in vivo studies of pulmonary platelet trapping.

Materials and methods

Platelet separation and labeling procedure

A method for platelet labeling with ¹¹¹In-oxine (Byk-Mallinckrodt, Holland) described earlier by Hardeman (1981) was used with modifications adapted for labeling of rabbit platelets. The separation and labeling procedure can briefly be described as follows. Whole blood (15 ml) was collected in a 20-ml syringe, containing 3 ml ACD-NIHA solution, transferred into a 20-ml injection vial, and was centrifuged at 130 g for 20 min (without brake). The platelet-rich plasma was transferred into another 20-ml vial. ACD-NIHA solution (0.7–0.9 ml ACD/10 ml platelet-rich plasma) was added to adjust the pH to 6.5.

The platelet-rich plasma was centrifuged at 500 g, for 10 min and left at rest for 30 min. All the plasma was then removed, and the remaining platelet pellet was resuspended in 1 ml phosphate buffered saline (pH = 7.4). ¹¹¹In-oxine (~6 MBq) was added, and the cell suspension was incubated for 15 min. The whole procedure was performed at room temperature (Christenson et al. 1983). The labeled platelet suspension was then finally reinfused into the animal.

Before injection the labeling efficiency was determined by centrifugation of 50 µl of the labeled cell suspension in a hematocrit capillary tube for 5 min at 700 g. The capillary tube was then cut just above the packed platelets. The labeling percentage was determined by comparing the activity in the cell and the plasma fraction measured in a NaI(Tl) well counter (Selektion 45-22).

Animal model

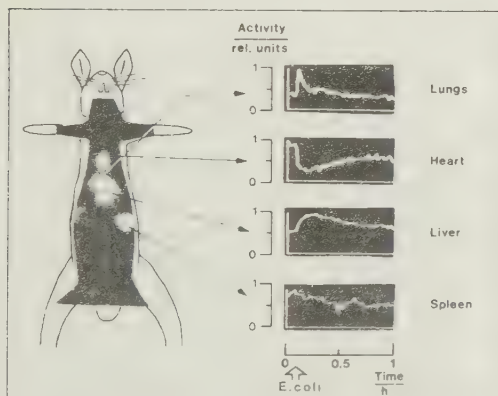
Nine rabbits weighing 2.1–2.6 kg were used (Table 1). The day after the platelet labeling an IV line was put in one ear for administration of about 40 mg/kg pentobarbitone sodium (ACO, Sweden) to anesthetize the animal. In the contralateral ear another catheter was put in for blood sampling (~1.5 ml) during the experiment. Thereafter 6 mg endotoxin *E. coli* (*E. coli* 0111:B4, Difco Laboratories) was given IV over 1 min. Blood samples for platelet counting and determination of blood activity were taken about 5 min before and 2 and 30 min after the *E. coli* injection from

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Table 1. The percentage of ^{111}In activity in the lungs and the heart before and after *E. coli* injection

Rabbit	Weight (kg)	Labeling efficiency (%)	Percentage activity (%)					
			Lungs			Heart		
			Before <i>E. coli</i>	2 min after <i>E. coli</i>	Relative change	Before <i>E. coli</i>	2 min after <i>E. coli</i>	Relative change
1	2.1	80	2.7	4.4	1.6	1.5	0.5	0.3
2	2.5	91	1.6	3.1	1.9	3.7	2.1	0.6
3	2.5	87	0.9	1.4	1.6	1.1	0.6	0.6
4	2.6	93	1.4	1.9	1.4	0.9	0.4	0.4
5	2.2	86	1.0	1.1	1.1	1.0	0.5	0.5
6	2.6	94	0.5	0.6	1.2	2.1	0.8	0.4
7	2.6	90	0.8	1.4	1.8	0.8	0.5	0.6
8	2.3	93	1.0	1.4	1.4	0.3	0.2	0.7
9	2.1	94	1.2	2.0	1.7	3.4	1.4	0.4
			Mean value 1.5 ± 0.3			Mean value 0.5 ± 0.1		

**Fig. 1.** The time-activity curves generated for different organs following IV injection of ^{111}In -labeled platelets. The induction of the endotoxin shock (indicated by arrow) is clearly seen in the lungs, heart, and liver curves

the ear not used for giving the above mentioned drugs (anesthesia and endotoxin). During the whole experiment the animals were breathing spontaneously

Blood activity measurements and platelet counting

The blood samples were collected in EDTA-tubes. A fraction (10 μl) was taken for platelet counting using phase contrast microscopy. The rest was weighed and measured for activity in an automatic sample-changing NaI(Tl) well counter

Scintillation camera technique

The anesthetized animal was put in a supine position under a scintillation camera (Searle Pho-Gamma III HP, Siemens) equipped with a 4,000-hole parallel-hole collimator, the day after the IV injection of the labeled platelets. An energy window of 25% was centered over the 245 keV full-energy peak. Images were stored on a computer (Gamma 11, Digi-

tal Eqp.) Sequential 60-s images were taken for 35 min. The first 5 min were used as reference values of the normal distribution of the ^{111}In -labeled platelets whereafter the *E. coli* was administered. After completion of the study, regions of interest, ROI, were selected over the heart, lungs, liver, and spleen and time-activity curves generated (Fig. 1)

Percentage uptake

From the count rate in the ROI $N(t)$, the real activity $A(t)$, could be calculated with the calibration factor $81.9 \pm 2.6 \text{ s}^{-1} \text{ MBq}^{-1}$ obtained in a rabbit phantom for the actual geometry using the formula,

$$A(t) = N(t)/81.9 \cdot \Delta t \quad (1)$$

where Δt is the measuring time interval.

In Table 1, the activity content in per cent of administered activity is given for the lung and the heart areas before and 120 s after the *E. coli* injection. The preinjection

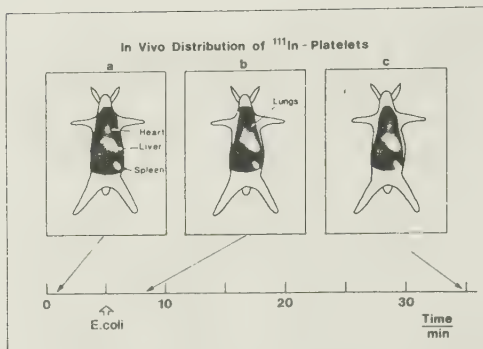


Fig. 2. Images of the in vivo distribution of ^{111}In -labeled platelets, before and after *E. coli* injection. Note the apparent change over the lung area in Fig. 2b compared with a and c

values were calculated by summing the count rate from 1 to 5 min after the start of registration and the postinjected values from 6 to 8 min. Table 1 also shows the relative change between these values.

Control studies

To exclude the possibility of an ^{111}In activity change in the lungs solely due to a redistribution in the total lung blood volume following the endotoxin shock, volume studies were simultaneously done with the ^{111}In -platelet study in one rabbit using $^{99\text{m}}\text{Tc}$ labeled red blood cells (Smith and Richards 1976) injected 5 min before the *E. coli* injection. A double isotope measurement was performed with one energy window centered over the 245-keV peak representing the platelet distribution and the other energy window centered over the 140-keV peak representing the red blood cell distribution.

To investigate the biokinetics of ^{111}In -oxine, only this substance was given IV in another experiment. The day after the injection a dynamic study was performed in the same way as for ^{111}In -labeled platelets combined with the *E. coli* injection.

Calculation of platelet sequestration

In Fig. 1 the time-activity curves are given for one of the experiments. The curve generated over the lung area represents the sum of both the increase of the platelet sequestration in the lungs and the decrease of circulating platelets in the lung blood. Before the *E. coli* injection, however, the activity in the lungs can be assumed to represent mainly the circulating lung blood activity. The change of lung blood activity follows the change of blood activity which is recorded over the heart, and the actual activity can be calculated knowing the ratio between the count rates over the lung and heart areas before the *E. coli* injection, k . With these assumptions the sequestration of platelets in the lungs $A_s(t)$ can be calculated by

$$A_s(t) = A_l(t) - k A_h(t) \quad (2)$$

$A_s(t)$ = Sequestered lung activity

$A_l(t)$ = Sequestered lung activity plus lung blood activity

$A_h(t)$ = Heart activity

Results

The labeling efficiency for each experiment is given in Table 1. The mean value for the whole series was $90\% \pm 5\%$ (± 1 SD). Figure 2a shows the scintillation camera image for the ^{111}In activity in one rabbit (No. 2) before shock, representing the 'normal' distribution of ^{111}In -labeled platelets in the rabbit. The heart, the spleen, and the liver were easily seen, whereas the lungs were impossible to distinguish.

Within about 2 min after the endotoxin had been administered the rabbit showed signs of respiratory distress. None of the animals died during the experiment.

Figure 2b shows the image of the same rabbit, 2 min after shock induction. An obvious change in the distribution of activity compared with the 'normal' picture could be seen. The lungs became clearly visible and the activity in the heart almost disappeared. Figure 2c shows the activity distribution in the rabbit about 35 min after the *E. coli* injection. Here the distribution had almost the same pattern as before the induction of endotoxin shock.

The change with time of the activity distribution after shock is also visualized on the curves in Fig. 1. The pre-shock recording showed stable activity over the heart and the lungs. After the *E. coli* injection a sudden rise was observed in the count rate curve over the lung area followed by a slow decrease to preshock values. The reverse pattern was registered for the count rate curve of the heart area. The recovery of the heart curve was much slower than the lung curve.

The count rate change in the liver resembled that for the lungs but the rate of change was much smaller. For the spleen there was a slow decrease of activity approaching a constant level after about 30 min.

The time-activity curves calculated from the count-rate curves with Eq. 1 are given in Fig. 3a for the lung and heart areas. The curves represent the mean values of the real activity for all the nine experiments. No error bars are included in the figure because of the difference between the exact size of ROIs for each experiment.

Based on the numbers in Fig. 3a the platelet sequestration was calculated from Eq. 2 and the results are given in Fig. 3b. The increase of the activity due to platelet en-

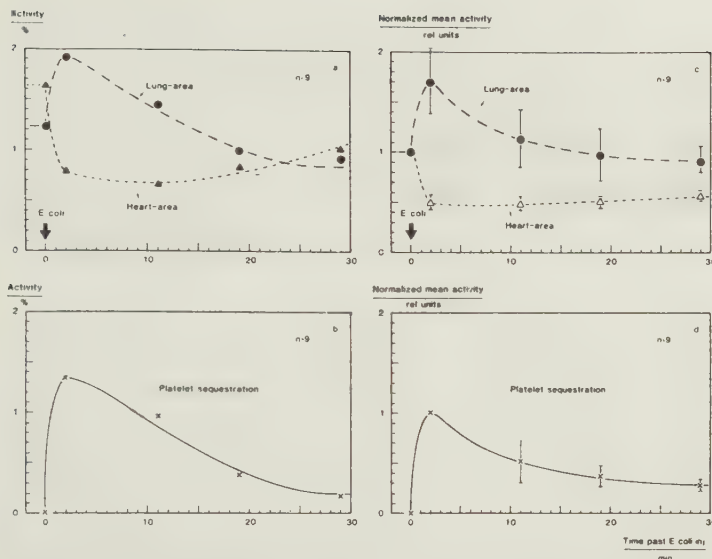


Fig. 3a-d. Time-activity curves and calculated PPT-values. a Mean time-activity curves of percentage activity in the lungs and heart. b Pulmonary platelet sequestration calculated from Fig. 3a and Eq. 2. c Normalized mean activity curves for the lungs and heart. d Platelet sequestration calculated from Fig. 3c and Eq. 2

trapment was obvious and the slow decrease of the activity approaching almost normal values within 30 min was clearly seen. In order to examine the reproducibility and the uncertainty of these registrations the differences in choice of ROI was corrected for by normalizing the activity curves to unity before the *E. coli* injection for all experiments.

The mean values of these normalized activity curves are given in Fig. 3c for the lung and heart areas. Included are error bars giving the standard deviation of the mean values. As can be seen, the errors were very small especially for the heart curves. The latter because the ROI for the heart was easily selected in the scintillation camera image. The errors were larger for the lung curve due to the differences in area choice between the experiments. From these normalized mean activity curves in Fig. 3c the platelet sequestration, $A_s(t)$, was also calculated for all the experiments. A value of 0.75 was obtained for k , being the ratio of the mean real activity over the lung area, 1.23%, and the mean real activity over the heart area, 1.64%, for all the experiments. The $A_s(t)$ values are given in Fig. 3d normalized to 1.0 at 2 min after the *E. coli* injection. The error bars ($\pm 1SD$) are small showing a good reproducibility for the measurements.

From Fig. 3a and b and Table 1 it can be seen that the activities were small in the lung and heart areas chosen, of the order of a few per cent of the injected activity. For the lungs the relative change between preshock values and 2 min after *E. coli* injection was 1.5 ± 0.3 (mean value for

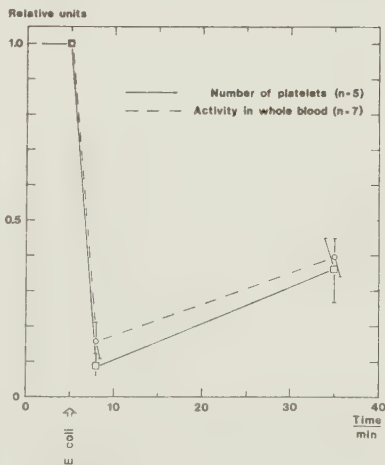


Fig. 4. The number of platelets and the radioactivity in whole blood, before and after injection of *E. coli*. Close correlation is apparent

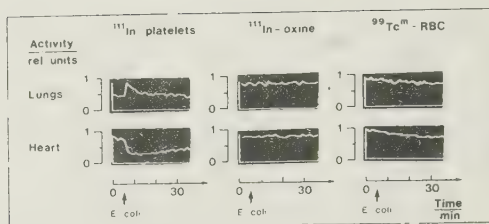


Fig. 5. Time-activity curves measured over the lungs and heart before and after *E. coli* injection for ^{111}In -labeled platelets, ^{111}In -oxine, and $^{99\text{m}}\text{Tc}$ -labeled red blood cells. The *E. coli* injection only affects the ^{111}In -platelets curves

all the experiments). The corresponding value for the heart was 0.5 ± 0.1 . Thus the change was very reproducible between all the experiments within a close interval.

The correlation between the number of platelets and the ^{111}In activity in peripheral blood is given in Fig. 4, where the two curves were normalized to unity for the values before shock induction. The platelet curve was the mean of five experiments and the activity curve the mean of seven experiments. As can be seen the two curves were almost identical and the difference was well within the error margins. Thus the decrease of activity recorded by the scintillation camera over the heart area must resemble the decrease in number of platelets in the same blood volume. The obtained count rate curves over the lung and heart areas for the control studies with ^{111}In -oxine and $^{99\text{m}}\text{Tc}$ -labeled red blood cells together with a typical ^{111}In platelet experiment are given in Fig. 5. In neither of the two control studies was any significant change in the count rate curves seen after the *E. coli* injection, as was present for the ^{111}In platelet study. This confirms that no volume change that might affect the PPT values occurred within the lungs or the heart after the shock induction.

Discussion

Endotoxin is believed to be a factor associated with irreversible shock and acute pulmonary pathology. The pulmonary response has been attributed to, among other things, release of vasoactive substances and mechanical blockage by platelet aggregates (Robb et al. 1972; Blaisdell and Lewis 1977; Demling 1980). A number of *in vivo* experiments have been made to demonstrate the sequestration of platelets in the lungs after endotoxin shock (Kux et al. 1972; Myrvold et al. 1977; Myrvold and Lewis 1977; Myrvold and Svalander 1977; Heideman et al. 1979; Myrvold 1980). In this study a noninvasive technique for *in vivo* dynamic studies of PPT has been developed. Images of rabbits with ^{111}In -labeled platelets taken with a scintillation camera have made it possible to study changes in the activity distribution before and after endotoxin-induced shock. A significant increase of activity was observed over the lungs and a simultaneous decrease over the heart following shock. Because almost all of the ^{111}In activity was bound to platelets, the activity measured for different organs was directly proportional to the number of platelets and thus PPT was registered *in vivo* following this experimental shock model.

Further evidence for registered *in vivo* PPT is the fact that when the lung activity increased, there was a simultaneous drop in the platelet count and an equally large drop in the activity of peripheral blood, indicating that most

of the activity was bound to the platelets. In the further course the parallel increase in the number of platelets and the activity in the blood indicated that platelet aggregation did not cause a release of free ^{111}In and thus only radioactivity bound to platelets was continuously recorded. The curve showing the heart activity was very similar from one experiment to the other and in the course of the experiment it was very similar to the blood activity curve. Therefore the heart values were used as a standard for the blood activity. This gives us the possibility to calculate the more 'exact' value of the platelet sequestration, PPT, because as shown the blood activity represents most of the background activity in the lung area. The time course of the PPT thus measured *in vivo* by our scintillation camera technique was very similar to other investigators' results from *in vitro* experiments (Heideman et al. 1979).

The activity change over the lungs was not caused by changed pulmonary blood volume as shown in the volume studies with $^{99\text{m}}\text{Tc}$ -RBC and in the experiment when ^{111}In -oxine was injected intravenously.

This study has shown that PPT and platelet sequestration in other organs can be detected, and that the dynamic course of platelet aggregation and disaggregation can be followed *in vivo* after endotoxin shock.

The method may therefore be of value in the study of the effect *in vivo* of different antiplatelet drugs. It is also possible that it may be useful in the clinic for the early detection of lung complications following shock from different causes. Further studies are under way to evaluate these possibilities.

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IN VIVO STUDY OF ENDOTOXIN INDUCED PULMONARY PLATELET
SEQUESTRATION. EFFECTS OF TWO INHIBITORY DRUGS

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Abstract:

Prostaglandin E₁ has earlier been shown to decrease pulmonary platelet trapping (PPT) following shock. This study was performed to compare the effect of prostaglandin E₁ and a new antiplatelet drug (Ticlopidine) on PPT in rabbits after endotoxin induced shock using a non-invasive model with In-111 labeled platelets. Following platelet labeling the rabbits were placed under a scintillation camera for continuous measuring of the activity distribution for 40 minutes. The first five minutes represented reference values, whereafter endotoxin E. coli was injected i.v. The following 2-4 minutes showed a sudden increase of radioactivity over the lungs and a simultaneous decrease over the heart, indicating PPT in the nontreated animals, followed by a slow decrease to almost preshock values during the following 30 minutes.

Animals receiving prostaglandin E₁ showed a significantly lower post shock activity peak in the lungs, while the corresponding peak in Ticlopidine treated animals did not differ from that in non-treated animals. In all groups endotoxin caused a decrease in platelet count, but it was significantly lower in the PGE₁ treated animals.

The results have shown that this diagnostic model for PPT is reliable and can be used for evaluation of the effect on platelet aggregation in vivo from different drugs.

Introduction

The role of platelets in the development of adult respiratory distress syndrome (ARDS) has been debated for many years and is still a matter of discussion. It is well known that different causes of shock, soft tissue trauma and major surgery often is followed by platelet aggregation and sequestration in the lungs (PPT) (1, 2, 3, 4). This makes the platelets suspected as being "precursors" to the later development of the above mentioned lung failure. Several investigators have developed experimental models to follow the reaction of platelets after shock from different causes, and to study the effect of different antiplatelet drugs on platelet aggregation in the lungs. The models have primarily been developed for in vitro animal experiments where blood or lung tissue was taken for ex vivo studies after specific drug treatment (5, 6, 7).

We have recently described an in vivo model for the study of PPT in the rabbit lung during shock caused by endotoxin (8).

The present study was designed to evaluate and compare the effect of two antiplatelet drugs on PPT, namely PGE₁ (Prostin^R VR, UpJohn, Sweden) which has a known effect on platelet aggregation and ticlopidine hydrochloride (Ticlopidine^R, Sanofi, France) a rather new antiplatelet drug which has a so far not entirely evaluated effect on platelet aggregation in vivo.

Material and Methods

Twenty rabbits weighing 2.0 - 2.6 kg were used for the experiments. From each animal 15 ml of blood was drawn for platelet labeling with In-111-oxine using a modified method described by Hardeman (9). From the labeled platelet suspension 50 μ l were taken for determination of the labeling efficiency and thereafter the labeled platelets were reinfused into the animal. The labeling procedure has been described more in detail in a previous report (8).

Approximately 6-8 hours after platelet labeling the animals were anaesthetized with about 40 mg/kg Bw pentobarbitone sodium (ACO, Sweden) through an i.v. line in the right ear. In the contralateral ear another i.v. line was inserted for administration of the the drugs during the experiment.

R

In the right femoral vein a small catheter (Venflon, Sweden) was inserted and used for blood sampling during the experiment.

The animal was placed in supine position in a specially moulded stand, to secure immobility during the experiment, under a scintillation camera (Searle Pho-Gamma III HP) for continuous measuring of activity in the animal (figure 1). For details of the scintillation camera technique see Thörne et al, 1984 (8).

Sequential 60 seconds images were taken for 40 minutes where the first 5 minutes represented reference values of the normal distribution of In-111-labeled platelets followed by 35 minutes of continuous measuring during and after drug administration. After 5.5 minutes shock was induced by intravenous injection of 2 mg/kg Bw endotoxin E coli (E coli 0111:B4, DIFCO LAB, U.S.A.) over one minute. After the completion of the experiment regions of interest (ROI) were selected over the heart and lungs and time activity curves were generated (figure 1).

The activity measured over the lung areas is the sum of the activity in platelets trapped in the lungs and those in the circulating lung blood. The activity over the heart is supposed to be proportional to the blood activity and thus pulmonary platelet trapping (PPT) is possible to calculate by subtracting the heart activity from the activity over the lungs using the following formula (8):

$$A_{s(t)} = A_{l(t)} - k \cdot A_{h(t)}$$

$A_{s(t)}$ = sequestrated lung activity

$A_{l(t)}$ = sequestrated lung activity plus blood activity

$A_{h(t)}$ = heart activity

k = ratio between the lung activity and heart activity before shock

The approximation of blood activity using the heart activity ($A_{h(t)}$) is easy to handle, but it should be more exact to use the values of platelet count or the In-111 activity value in a blood-sample in the above mentioned formula. Therefore these values were also used as controls to evaluate the accuracy of the above mentioned approximation.

The animals were divided into four groups.

Group A, endotoxin with no treatment (4 animals)

Group B, endotoxin with Ticlopidine (7 animals)

Group C, endotoxin with prostaglandin E₁ (5 animals)

Group D, prostaglandin E₁ but no endotoxin (4 animals).

The animals in group B recieved 150 mg/kg Bw Ticlopidine dissolved in 10 ml water daily via a temporarily inserted gastric tube starting three days before the experiment. The other animals were given the same amount of water thus handled the same way.

The animals in group C and D recieved prostaglandin E₁ i.v. through the catheter in the left ear, - 50µg/kg Bw continuously over two minutes, starting after the first five reference minutes. PGE₁ was thus given 30 sec before, during and 30 seconds after the infusion of endotoxin.

During the experiment, blood samples (approx. 3 ml totally) were taken 5 minutes before, 2 minutes and 30 minutes after shock for determination of hematocrite, platelet count and In-111 activity in the blood. Platelet counting was done using phase contrast microscopy and blood activity was measured in an automatic sample-changing Na I(Tl) well counter.

No animals recieved ventilatory support during the experiment. To keep the i.v. -lines open small amounts of saline were given.

After the experiment all rabbits were sacrificed with an overdose of pentobarbitone sodium.

Statistical analysis were made with Mann-Whitney rank-sum test.

Results

Platelet labeling efficiency for all animals ranged 64-95% with a mean value of $82 \pm 7\%$ (± 1 .SD).

Hematocrite did not change significantly during the whole experiment in any of the animals.

Administration of endotoxin in groups A - C was almost immediately followed by signs of shock i.e. shallow fast breathing, pale nose and pale mucous membranes in the mouth and disappearance of palpable pulse in the femoral artery.

Animals receiving only PGE₁ did not show any signs of shock.

The platelet count decreased significantly three minutes following endotoxin injection but the decrease was less prominent in the prostaglandin E₁ treated group C ($P < 0.05$) seen on figure 2 a where the platelet count is normalized to unity. The platelet count in animals treated with Ticlopidine did not significantly differ from the untreated group A. In group D there was no significant change in platelet count over the whole experiment.

Figure 2 b shows that there was a similar change in the ¹¹¹In activity in the blood with a significant decrease in blood activity three minutes following shock induction but significantly ($P < 0.05$) less dramatic in animals treated with prostaglandin E₁ (group C). There was no effect on blood activity in the Ticlopidine treated group B. The platelet count and blood activity values at 30 minutes did not differ significantly in any of the shock groups.

Treating rabbits with PGE₁ without endotoxin shock (group D) did not affect the blood activity.

In table I the platelet count in each group is seen.

Figure 1 shows time-activity curves generated for the heart and lungs in three animals from group A, C and D respectively. The change in activity over the two selected ROI following shock is obvious with a fast decrease in the heart activity and a simultaneous increase over the lungs, which is less prominent in the PGE₁ - treated group. No significant change in the heart and lung activity was recorded in the nonshock group D during the experiment.

The count rate over the lungs and heart in each experiment is normalized to unity and the mean values in each shock group is illustrated in figure 3. Figure 4 shows the pulmonary platelet sequestration ($A_{s(t)}$) calculated from the values in figure 3 using the equation.

From figure 3 and figure 4 a significant increase in lung activity after endotoxin shock is seen within 2-4 minutes followed by a slow decrease to almost preshock values. There is no significant difference between the groups in time lag till the activity peak is reached. The activity maximum is significantly

lower in the PGE₁ treated group C ($p < 0.02$) and this significant difference is the same in both the normalized curves and the corrected curves where the equation is used. At 10 minutes postshock there is still a significantly lower activity in the prostaglandin treated group ($p < 0.05$) when correction for the background activity is performed, but after 20 minutes the difference is not significant.

The Ticlopidine treated rabbits do not at any time differ from the untreated Group A.

The change in In-111 activity in the heart was significant in group A-C, but there was no significant difference between the groups. In the equation $A_{h(t)}$ represents the heart activity approximated to blood activity. The platelet count and blood activity values normalized to unity were also used in the formula for calculating the pulmonary platelet sequestration ($A_{s(t)}$). This did not change the significance ($p < 0.02$ in all cases) indicating that heart activity is an acceptable approximation of the blood activity.

Discussion

ARDS is often caused by sepsis (5, 7, 10), but the etiology is still obscure and a matter of discussion. The role of platelets and leucocytes in the early phase of the development of respiratory insufficiency following sepsis is accepted as is the release of vasoactive substances such as serotonin and prostaglandin after the initial cell aggregation (11, 12). Different animal models have been set up to simulate clinical sepsis and to study pulmonary platelet trapping and the effect of different drugs. No real good "sepsis" models are available but using endotoxin E.coli intravenously is often used to simulate a "sepsis" situation. The platelet studies are usually done on blood samples ex vivo, or, after radiolabeling platelets, by performing lungbiopsis or simply by removing the whole lung at a certain time after shock and measure the radioactivity in the tissue (5, 6, 7, 8).

We have earlier shown that dynamic studies on PPT are possible to do by using Indium-111 oxine labeled platelets (8), thus making it possible to study the reaction of platelets over a period of time during shock in vivo. This experiment has clearly shown that earlier studies done this way are reproducible. Endotoxin shock causes an increase in activity over the lungs and a simultaneous decrease over the heart. At the same time there is a decrease in the number of circulating platelets. As In-111 labeled platelets

were used this indicates that the activity increase in the lungs is caused by platelet trapping, since earlier experiments have shown that all activity is bound to the platelets and that platelet aggregation does not "release" the activity (13, 14, 15). Favouring this explanation is the fact that the blood activity decreases at the same time as the decrease in platelet count and the increase of lung activity. These results are in agreement with our earlier experiments (8).

PGE₁ is known to be a potent inhibitor of platelet aggregation (7, 16) which is confirmed by this experiment. The most evident result in this study is the fact that it is possible in vivo to register this inhibitory effect of prostaglandin E₁ in this very strong platelet activating model.

The inhibitory effect of prostaglandin E₁ is significant seen on the count rate curve from the lungs without any correction (i.e. subtracting the activity from platelets in circulating blood), but is even more significant after correction by using the heart activity (approximated to blood activity) in the equation. The blood activity is easily calculated from the platelet count or In-111 activity in a blood sample, but using the later values in the formula did not alter the results. The antiplatelet effect of PGE₁ is also obvious regarding the platelet count and the activity in peripheral blood - where the decrease in these parameters is significantly lower in the PGE₁ treated animals.

Ticlopidine - was also tested (17, 18, 19, 20). It is said that this drug has its effect only after oral administration thus indicating that the active substance is probably a metabolite of the drug (17, 20). It has also been found that especially in rabbits the concentration in blood of active drug is reached slowly, but the effect of the drug is expected after 48 hours. Thus our animals treated over 72 hours should have reached a sufficient blood concentration (17, 19, 20). Finally, the drug is suggested to have irreversible effect on the platelets, but the pharmacology is still not fully explained (17, 19, 20).

Our experiments did not show any effect of Ticlopidine on PPT. Nor did we find any significant difference in the platelet count or blood activity in the Ticlopidine treated group.

This study confirms the positive effect of PGE₁ on platelet aggregation and PPT. The failing effect of Ticlopidine could be explained by the fact that this shock model, using an extremely potent substance - endotoxin E.coli - for induction of platelet aggregation is simply "overwhelming" the expected positive effect of Ticlopidine. The inhibitory response of Ticlopidine is supposed to be dependent on the strength of the stimulus and probably has different influences on aggregation induced by various agents (17, 20).

Another possibility is species differences, where rabbits perhaps need a larger dose and/or a prolonged treatment than other animals (17, 20).

Finally, a possible explanation is that Ticlopidine has little or no effect on platelet aggregation in vivo. Further studies have to be done to secure the effect of this drug in vivo.

In conclusion this study demonstrates a shock model that makes it possible to study PPT. It also confirms the known positive effect of PGE₁ in vivo on PPT in endotoxin shock, while Ticlopidine does not seem to have any effect on platelet aggregation in this model.

In our opinion the model is usable for further studies on PPT and the results also indicate the possibilities to use it for evaluation of the effect of other drugs on platelet aggregation in vivo.

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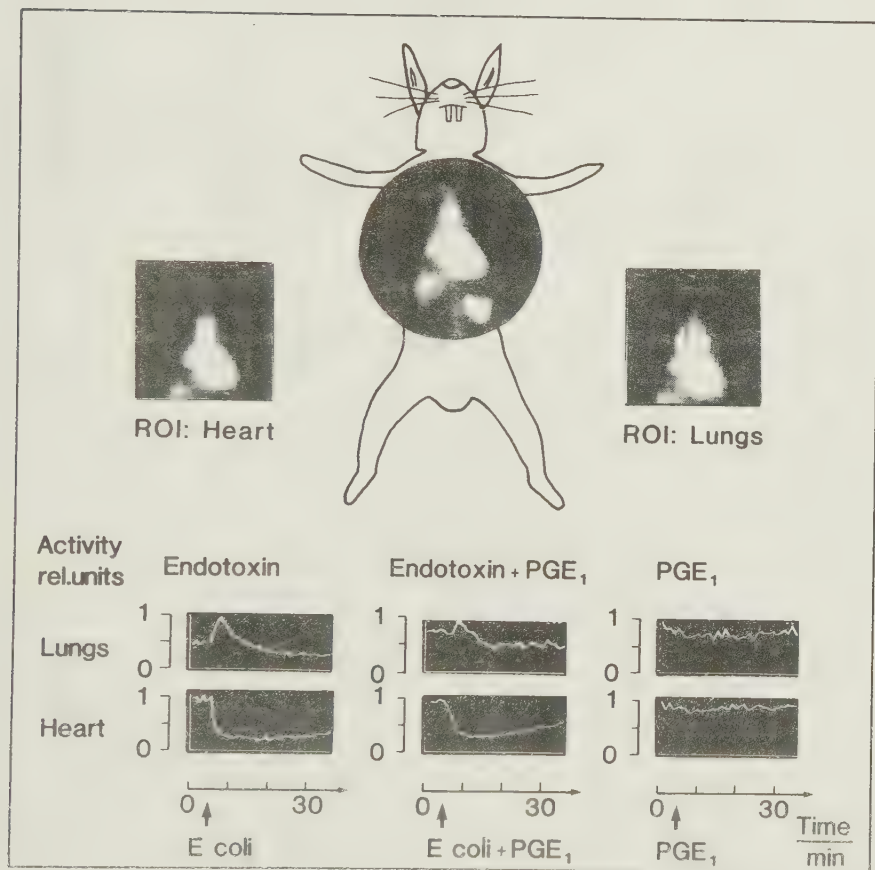


Figure 1. The scintillation camera field of view and time-activity curves generated for the heart and lung regions in three animals from group A, C and D respectively.

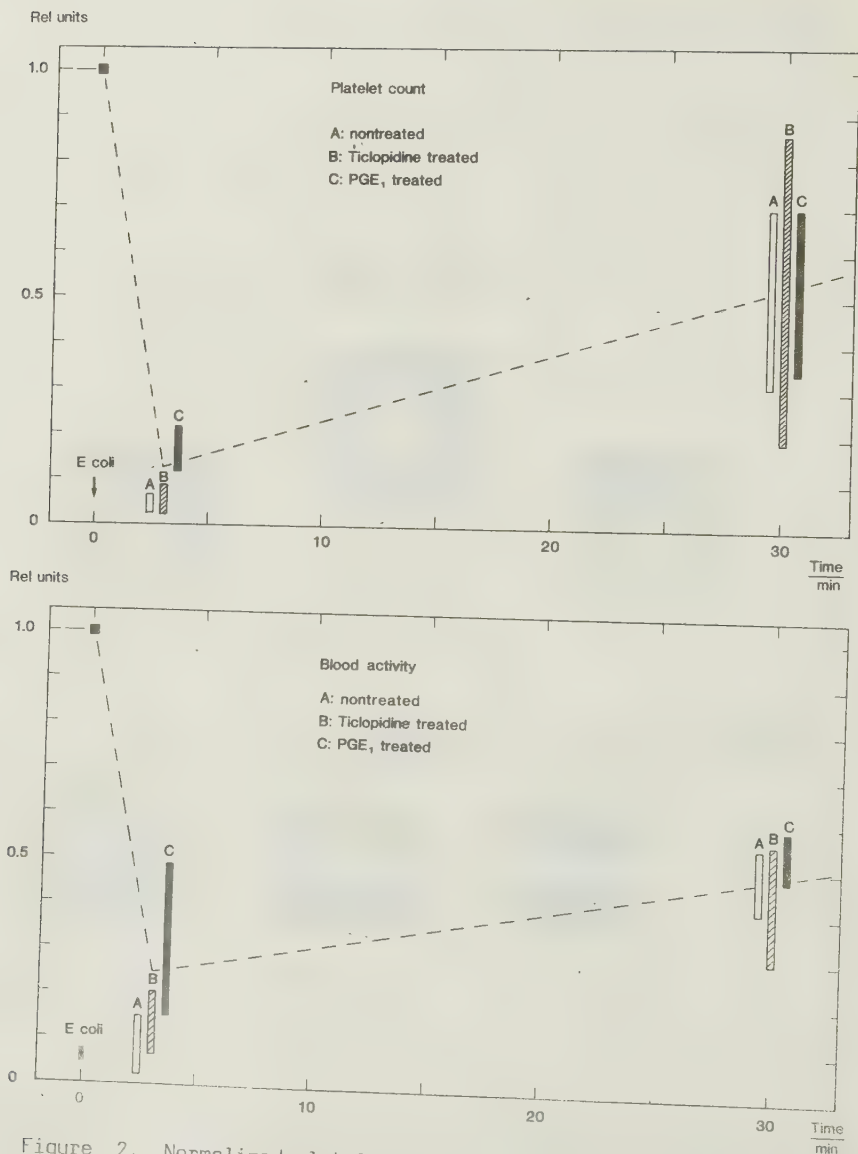


Figure 2. Normalized platelet count (a) and blood activity (b) for the three groups A, B and C before and after *E. coli* injection. The bars indicate range. The decrease in the platelet count and blood activity was less prominent ($P < 0.05$) in the PGE₁-treated animals (C) than in group (A) and (B).

TABLE 1. Number of platelets per mm^3 in the blood samples from the four groups.

Group	n	Platelets (10^3 mm^{-3})			
		Preshock		Shock	
		0 - 5 min	+ 3 min	+ 25 min	
Endotoxin	(A) 4	458 $\pm$ 90	18 $\pm$ 6	286 $\pm$ 84	
Endotoxin + Ticlopidine	(B) 7	544 $\pm$ 146	20 $\pm$ 9	275 $\pm$ 162	
Endotoxin + PGE ₁	(C) 5	467 $\pm$ 119	80 $\pm$ 16*	239 $\pm$ 77	
PGE ₁	(D) 4	599 $\pm$ 82	558 $\pm$ 70	596 $\pm$ 109	

All values mean $\pm$ 1 S.D.

* $P < 0.05$ (Mann-Whitney U-test) compared to control group A.

Normalized mean activity
relative units

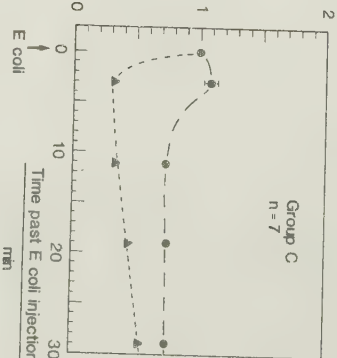
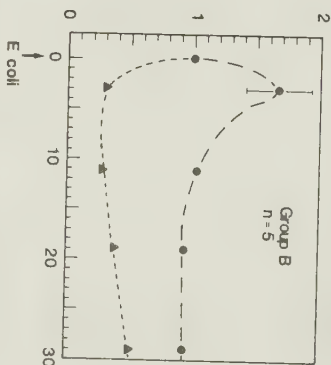
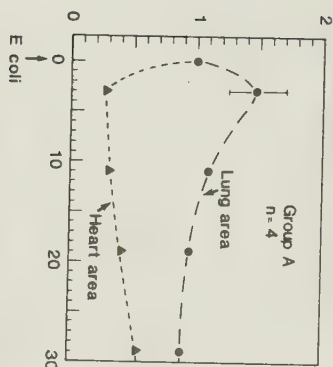
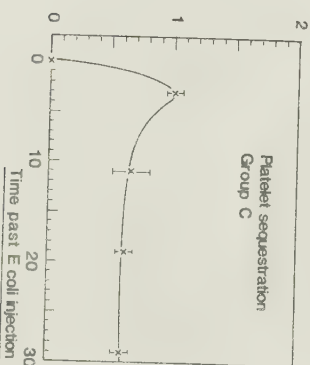
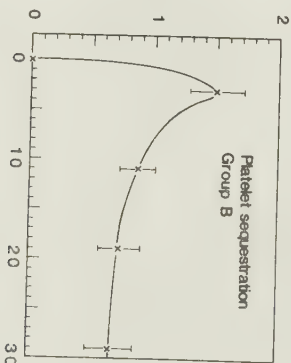
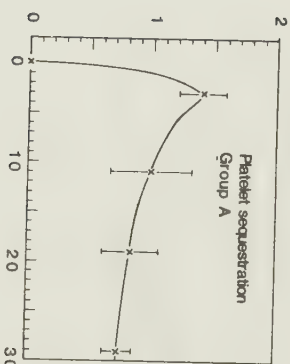
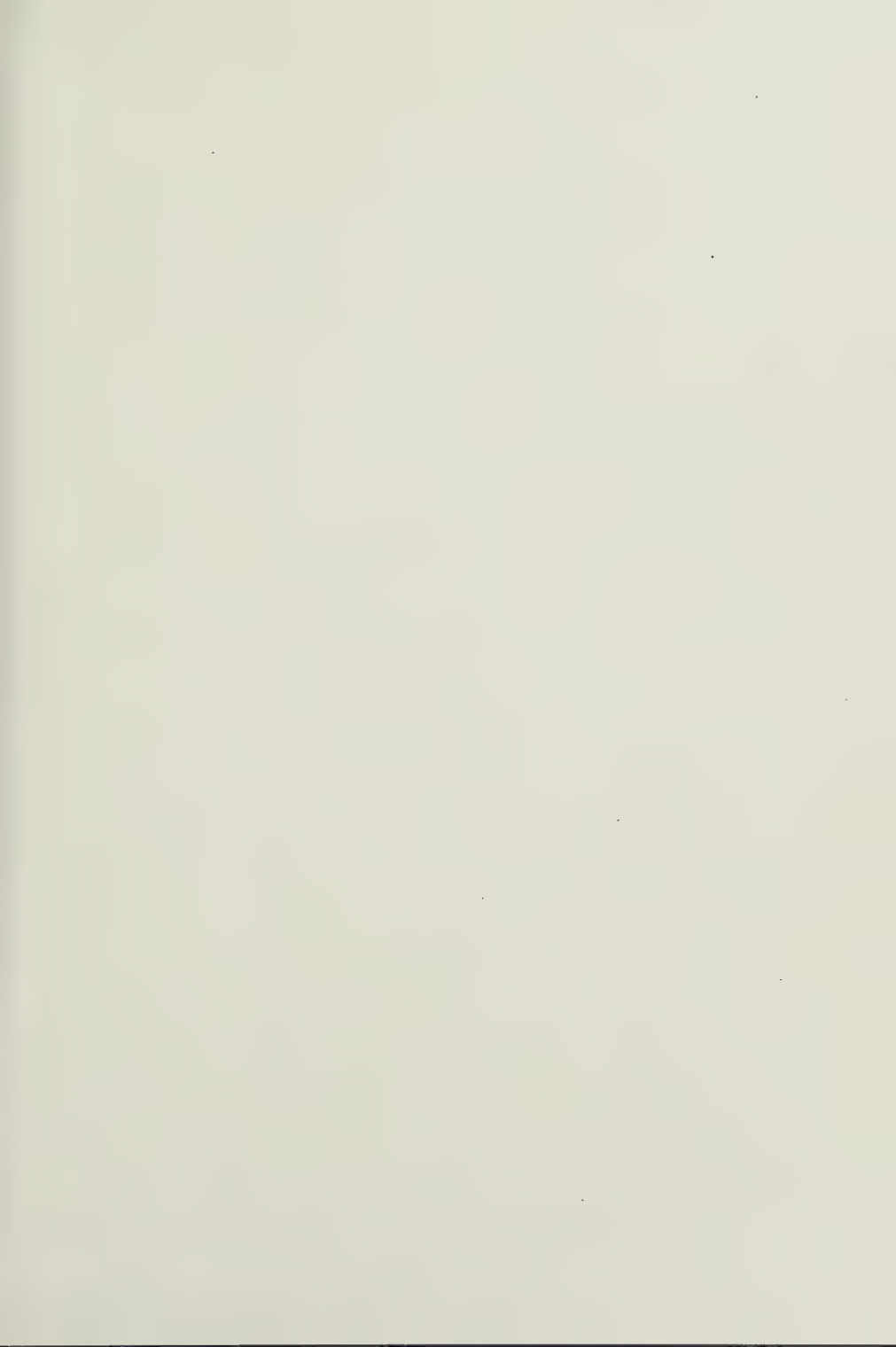


Figure 3. Normalized mean time-activity curves for the lungs and heart in group A, B and C. The pulmonary activity increase after shock is significantly lower in the PGF₁-treated animals, group C.

Normalized mean activity
relative units







EARLY POSTTRAUMATIC PULMONARY PLATELET TRAPPING AND ITS POTENTIATION
BY ORAL PRETREATMENT WITH ALCOHOL

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INTRODUCTION

The adult respiratory distress syndrome (ARDS) remains a major complication to traumatic shock and sepsis in surgical patients and its underlying causes are still not known. The syndrome has been associated with pulmonary trapping of platelets and granulocytes (1), which are believed to cause pulmonary microvascular obstruction resulting in increased pulmonary vascular resistance (2), either mechanically by obstruction of small vessels (3) or chemically by the release of vasoactive substances (4). These events may affect pulmonary endothelial cells and increase vascular permeability (5), which is a well documented major clinical finding in ARDS.

As a response to endotoxin infusion, pulmonary platelet trapping (PPT) is known to occur almost immediately (6). PPT is, on the other hand, thought to be a gradual process during hours after traumatic shock (7). A discrepancy in the timing of PPT after endotoxic and traumatic shock could be a reflection of different etiological mechanisms. But, the early phase of PPT in traumatic shock has hitherto not been conclusively elucidated, and since at least one mechanism for PPT could be an activation of platelets at the trauma site, this period needs to be studied.

The aim of the present study was to follow the development of PPT after a standardized soft tissue trauma in anaesthetized pigs. The continuous in vivo measurements of the platelet biokinetics was enabled by a recently developed scintillation camera method using radiolabelled platelets (8). In the study, oral alcohol pretreatment which increases PPT (9), was also used in order to investigate possible modifications in individuals, influenced by alcohol, a practical situation frequently met in the emergency room.

MATERIAL AND METHODS

Ten domestic pigs weighing 25-30 kg were studied. They were all fasted over night. In the morning 100 ml of blood was withdrawn via an indwelling catheter placed in the jugular vein. The platelets were labelled with 20-25 MBq of ^{111}In -oxine (8). The labelled platelets were then reinfused. The labelling efficiency was determined by comparing the activity in plasma with the activity in the cells separated by centrifugation in a haematocrit tube.

Approximately 4-6 hours after the reinfusion of the labelled platelets, the animals were anaesthetized with 12.5 mg/kg bw thiopentalum (Pentothal[®], Abbott, Sweden) intravenously. The animals were intubated and ventilated with N_2O and O_2 using a mechanical ventilator (Siemens-Elema, Sweden). Anaesthesia was maintained with repeated doses of fentanylum (Leptanal[®], Leo, Sweden) and alcuronium chloride (Alloferin[®], Hoffman-La Roche, Switzerland). A catheter was placed in the carotid artery and connected to a transducer for continuous blood pressure measurements. A gastric tube was passed into the stomach. The animals were then fastened in a specially molded plastic stand, to secure an unchanged position under the scintillation camera during the whole examination. The scintillation camera (Searle Pho-Gamma III MP, Siemens) was equipped with a parallel holes collimator. Images of the thoracic and upper abdominal area were stored in a computer (Gamma 11, Digital Equipment, USA). The total registration time was 140 minutes where sequentially 60 s images were recorded. The experimental set-up is illustrated in Figure 1.

With the animals in a circulatory steady state basal values were recorded: Via the gastric tube 6 animals received 15 ml/kg bw 40 % ethanol in saline

and 4 pigs a corresponding value of saline, both as a single dose. The gastric tube was then removed. Ninety minutes after the administration of alcohol or saline the animals were subjected to a standardized blunt soft tissue trauma against each hind limb with 100 blows of a rubber mallet for approximately 2 minutes. All measurements were recorded immediately before trauma, 3, 10 and 30 minutes after the trauma. At each moment of measurements a blood sample was withdrawn for platelet counting, for determination of haematocrit and for measurement of radioactivity. After the experiment the animals were sacrificed.

After completion of the scintillation camera registration regions of interest (ROI) over the heart as well as over the lung area were selected and time-activity curves generated. As the activity recorded over the lungs represents the sum of activity from platelets sequestered in the lungs and platelets circulating in the blood, PPT was calculated using the following formula (8):

$$A_S(t) = A_L(t) - k \cdot A_H(t)$$

$A_S(t)$ = sequestered lung activity

$A_L(t)$ = sequestered lung activity + blood activity

$A_H(t)$ = heart activity (approximately blood activity)

k = lung/heart activity ratio before trauma

Statistical methods used were the Mann-Whitney rank sum test for comparisons between groups and the Friedman test and paired t-test for within group analyses.

RESULTS

The labelling efficiency did not differ between the two groups ($71.1 \pm 4.7\%$ and $77.3 \pm 1.6\%$ respectively). During the period of 90 minutes prior to the trauma no measured variables were changed in either group. After the soft tissue trauma, mean arterial blood pressure was decreased in both groups and remained low during the thirty minutes observation period (Table I). No animals died during the experiment. Autopsy revealed haematoma and soft tissue injury. No fractures or any rupture of large vessels were found at the trauma sites.

At the measurements 3 minutes after the trauma, the lung activity was significantly increased in both groups of animals (Figure 2). Posttraumatic PPT was, however, significantly higher after alcohol pretreatment compared with control animals given saline. At the same time, the number of circulating platelets (Table I) as well as the radioactivity in peripheral blood were lowered (Figure 3).

Ten minutes after trauma, the activity over the lungs was lower than the 3-minute values. It remained higher in alcohol treated animals than in controls. Peripheral platelet counts were normalized in animals pretreated with saline but stayed below pre-trauma values in alcohol treated animals (Table I). Radioactivity in whole blood stayed below pre-trauma values in both groups (Figure 3).

Thirty minutes after trauma, a further decrease in lung activity from trapped platelets was measured, but the values still remained higher than prior to trauma. The platelet count was still reduced compared to pre-trauma

in the alcohol treated group while radioactivity of whole blood in both groups was still lowered (Table I, Figure 3). The scintillation camera measurements of lung activity in one animal from each group related to the time after trauma are given in Figure 4.

DISCUSSION

The cause of lung damage in connection with traumatic shock has been a matter of debate for many years and it is still a most controversial subject. The interest has been focused on the behaviour of blood platelets in pulmonary vessels and in studies using radiolabelled platelets the presence of an increased number of platelets in the lung was revealed after trauma (10). These studies demonstrated PPT after a couple of hours which together with previous reports on the subject (11) support the assumption of PPT as an important etiological factor for development of posttraumatic pulmonary insufficiency. To be able to form a basis for adequate early treatment in patients subjected to major trauma, it is vital to know the timing of the platelet trapping in the lungs after trauma.

In this study, a newly developed in vivo scintillation camera technique with ^{111}In -labelled platelets has been used. This method made it possible to follow dynamic changes of platelet trapping in the lung. Since the technique allows the study of relative changes in radioactivity, it was of the utmost importance that the position of the animal related to the gamma camera was precisely the same before and after the trauma. Therefore, a specially molded stand was constructed and identification marks on the animal were used to relate the animal to the gamma camera.

The increase of ^{111}In activity over the lungs, 3 minutes after trauma, was associated with a simultaneous decrease in activity over the heart, i.e., blood activity, as well as in the number of circulating platelets in peripheral blood. This clearly showed that platelets are being trapped in pulmonary vessels shortly after this moderate soft tissue trauma. Related to previous investigations of PPT, that have shown a gradual trapping occurring during several hours after the trauma (7, 12), the present result with a high initial trapping which was decreasing already at 10 and 30 minutes, was surprising. Earlier studies utilized, however, different techniques with repeated lung biopsies or removal of the lung at certain times after the trauma. This was circumvented in this study as the technique allowed dynamic in vivo observations, and the present results are, in accordance with an earlier study in which pulmonary retention of circulating microaggregates following soft tissue trauma was indicated (9). In that study, the screen filtration pressure (SFP) of blood was decreased after passage over the pulmonary circulation at about the same early stage as was found in this study.

Early PPT following soft tissue trauma might be caused by activated platelets which form microaggregates, after they have been exposed to subendothelial structures (11). These activated platelets become "sticky" and may on these grounds be trapped in the lungs, since this is the first capillary bed they will reach. But they could also form microaggregates which are too big to pass capillaries and result in mechanically obstructed pulmonary microvessels (3). Platelets trapped in the microvessels also challenge the assumption of a vascular obstruction and uneven perfusion secondary to the release of vasoactive substances (4). The results in this study with evidence of an early platelet trapping in the lungs do, however, not eliminate other

possible mechanisms behind the disturbed pulmonary perfusion after trauma. One such mechanism is that of central nervous origin (13). Nervous reflexes are instant and a combined effect of central nervous reflexes causing vasoconstriction and altered platelet morphology and function might both be of importance for the development of this complex pulmonary insufficiency.

The combination of a vasopressor function in lung vessels and altered characteristics of platelets for the development of ARDS could also be supported by the more profound PPT noted in alcohol treated animals. Ethanol is known to exert vasopressor activity on lung vessels (14) and is also known to increase platelet aggregability at high concentrations (15). Consequently, both mechanisms of ethanol potentiate PPT in connection with soft tissue trauma. The increased PPT in animals under the influence of ethanol is, however, in contrast to studies indicating reduced PPT after pretreatment with CNS depressants (16). The central nervous depressant effect of ethanol was obviously not sufficient to eliminate the documented effect of ethanol on lung vessels and platelet function.

It is concluded that pulmonary platelet trapping occurs within minutes after soft tissue trauma. PPT is markedly increased in animals traumatized while under the acute influence of alcohol. Thus, ethanol potentiates PPT and constitutes an increased risk for pulmonary dysfunction after trauma, which underlines the need of an early institution of aggressive cardio-pulmonary support in these patients.

SUMMARY

Soft tissue trauma is associated with platelet aggregation and sequestration in the lungs. This is believed to be an early step in the later development of adult respiratory distress syndrome (ARDS). In the present experiment using a new method for in vivo dynamic studies of platelet sequestration we wanted to evaluate the effect of soft tissue trauma on pulmonary platelet trapping (PPT) in pigs and the influence of acute ethanol intoxication.

The results show that significant PPT is registered within minutes of the trauma and that ethanol significantly increases platelet sequestration in the lungs. This indicates an increased risk for posttraumatic pulmonary problems in alcohol intoxicated trauma victims.

ACKNOWLEDGEMENTS

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MAP, mm Hg	0 min	90 min	Trauma + 3'	Trauma + 10'	Trauma + 30'
Saline	157 $\pm$ 12	152 $\pm$ 5	118 $\pm$ 16*	129 $\pm$ 5*	119 $\pm$ 4*
Ethanol	154 $\pm$ 17	136 $\pm$ 24	86 $\pm$ 18**	101 $\pm$ 17*	95 $\pm$ 20*
Platelet count, $\times 10^9/l$					
Saline	418 $\pm$ 63	430 $\pm$ 69	281 $\pm$ 37**	346 $\pm$ 33	434 $\pm$ 61
Ethanol	354 $\pm$ 39	384 $\pm$ 40	245 $\pm$ 32**	287 $\pm$ 40*	319 $\pm$ 40*
PPT, relative units					
Saline	0	0.06 $\pm$ 0.02	0.49 $\pm$ 0.07*	0.16 $\pm$ 0.03**	0.09 $\pm$ 0.04
Ethanol	0	0.07 $\pm$ 0.02	0.89 $\pm$ 0.05***	0.44 $\pm$ 0.08**	0.26 $\pm$ 0.07*
Blood activity, relative units					
Saline	1	0.90 $\pm$ 0.02	0.80 $\pm$ 0.04*	0.85 $\pm$ 0.03**	0.87 $\pm$ 0.03
Ethanol	1	0.95 $\pm$ 0.02	0.81 $\pm$ 0.05*	0.88 $\pm$ 0.05	0.93 $\pm$ 0.03

Table I.

Mean arterial blood pressure (MAP), platelet count, PPT and radioactivity of blood in pigs pre-created with saline (n = 4) or ethanol (n = 6) expressed as mean $\pm$ SE. Within group statistical analysis of values at 90 min and post trauma values. * p < 0.05, ** p < 0.01, *** p < 0.001.

SUMMARY FOR TABLE OF CONTENTS

With a new radio-isotope technique pulmonary sequestration of platelets was dynamically studied following soft tissue trauma to pigs. Platelets were trapped in the lungs within minutes of the trauma and platelet trapping was significantly increased by oral pretreatment with alcohol.

Key words: Platelets, Lung, Trauma, Alcohol.

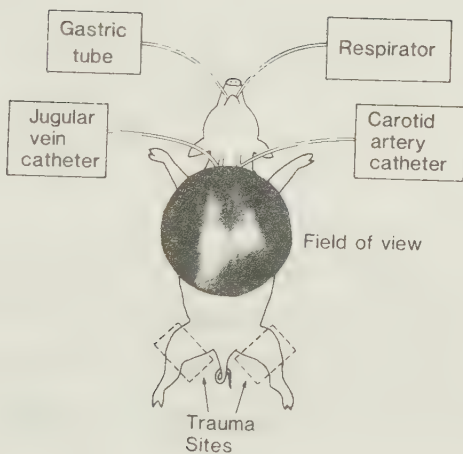


Figure 1 Experimental set-up and scintillation camera field of view.

Normalized mean activity

rel units

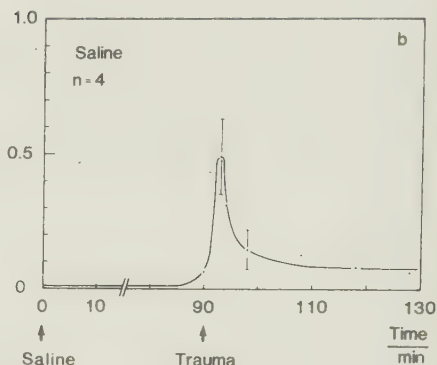
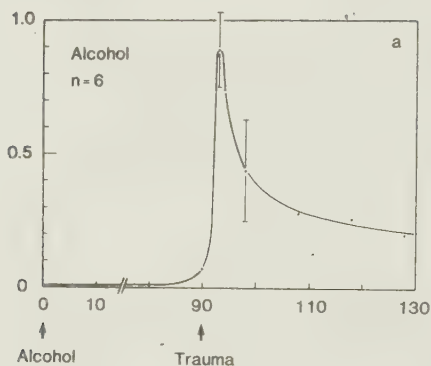


Figure 2 Pulmonary platelet trapping (PPT) $\pm$ 1SD before and after soft tissue trauma in animals pretreated with alcohol (a) or saline (b). PPT was significantly higher 3 and 10 minutes after trauma ($p < 0.02$) in animals pretreated with alcohol.

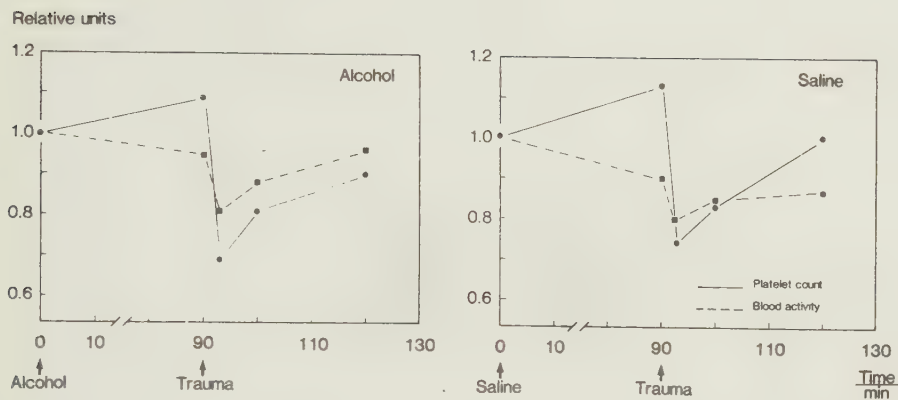


Figure 3 Peripheral platelet count and blood activity in animals pre-treated with alcohol or saline prior to a standardized soft tissue trauma. Arbitrary units related to time 0.

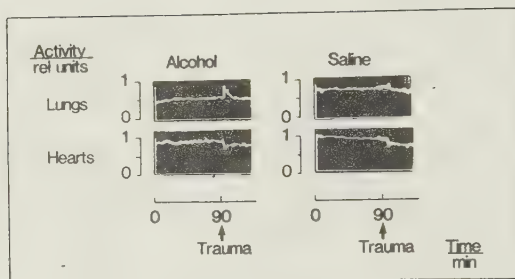
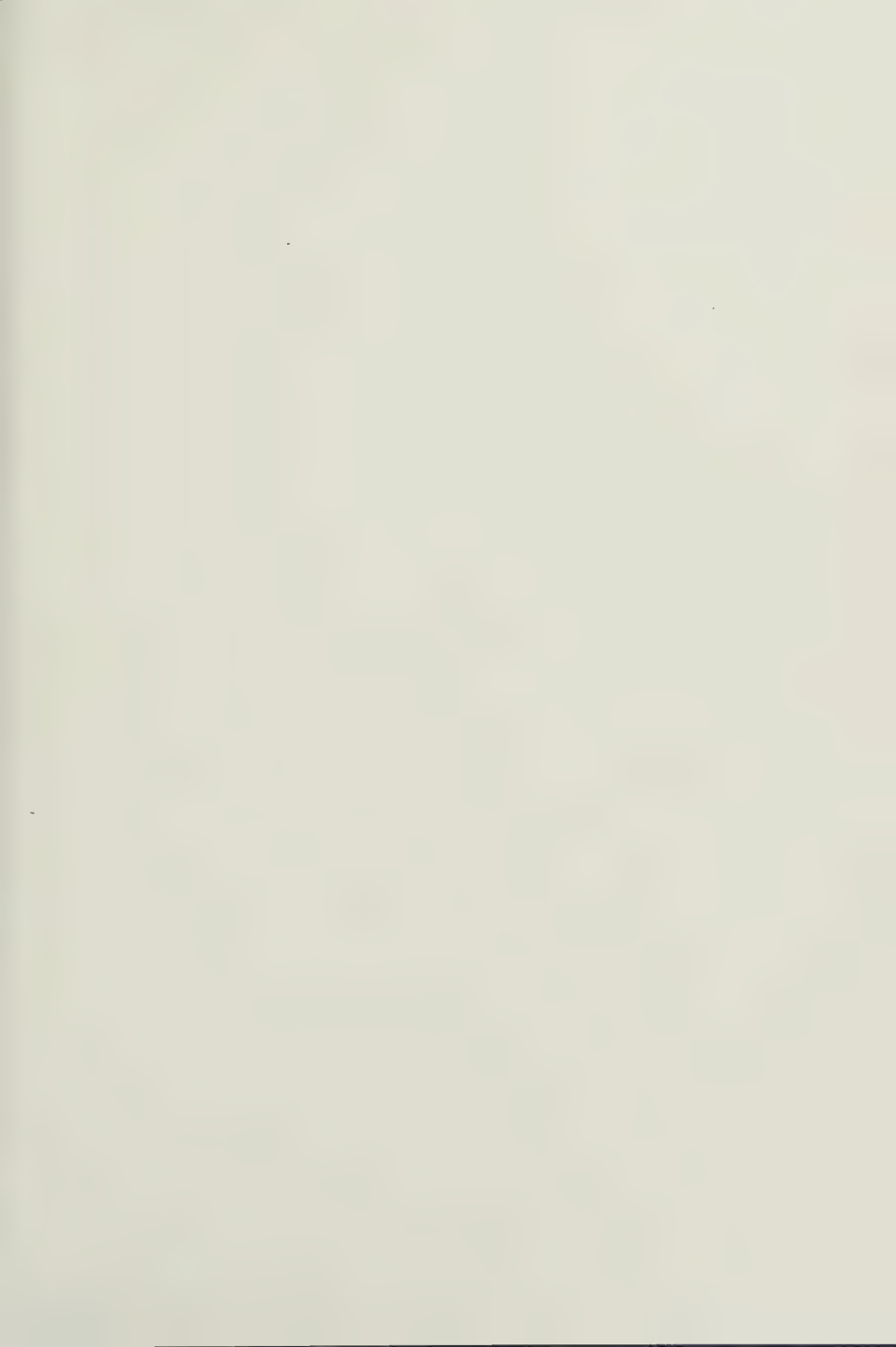


Figure 4 Time-activity curves generated for the heart and lung regions from one animal pretreated with alcohol and one pretreated with saline.



EARLY POSTTRAUMATIC CHANGES OF HEMODYNAMICS AND PULMONARY VENTILATION
IN ALCOHOL PRETREATED PIGS

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Key words: alcohol, central hemodynamics, lung mechanics, trauma.

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Abstract

Time relations between trauma, pulmonary and systemic circulation and lung function were studied in pigs. Eleven animals (b.w. 25-30 kg) were investigated under balanced anaesthesia. Ventilation was mechanically controlled. Hemodynamics, pulmonary ventilation and gas exchange were followed. Seven animals were pretreated with 40% ethanol in saline and four with saline only. Ninety minutes after the ingestion of alcohol or saline, the animals were subjected to a standardized soft tissue trauma. Cardiac output decreased significantly 2 minutes after trauma and remained low in both groups throughout the observation period of thirty minutes. Pulmonary vascular resistance was significantly increased in the alcohol pretreated group but was virtually unchanged in the control animals. Systemic vascular resistance was similarly reduced in the two groups. Total compliance was somewhat lower in alcohol pretreated animals and 10 minutes after the trauma arterial oxygen tension was significantly lower in the alcohol group than in control animals. Carbondioxide elimination was reduced after trauma in both groups. It is concluded that pulmonary vascular response increased and that total pulmonary compliance is somewhat decreased shortly after trauma in the alcohol group while gas exchange is almost unchanged. The results indicates a negative interaction between alcohol and trauma.

Introduction

It has previously been shown that intoxicated patients withstand acute blood loss poorly (1) and even minor trauma in such patients result in profound hypotension with bradycardia (2, 3). It has also been suggested that alcohol has a direct cardiodepressant effect that could be responsible for the increased mortality noted in experimental studies on dogs (4) and rats (5) after blunt chest trauma. In conformity with those results Elmér et al (6) also found an increased mortality in alcohol intoxicated pigs after a standardized soft tissue trauma. In that study cardiac output did not recover after trauma in the alcohol pretreated animals and indirect evidence of pulmonary platelet trapping was found.

It has also recently been shown by Thörne et al (7) that ¹¹¹Indium labelled platelets were trapped in the lungs immediately after a soft tissue trauma which further emphasizes that platelets are involved in the development of adult respiratory distress syndrome (ARDS). The effects of trapped platelets in pulmonary microvessels is, however, debated. Platelets may cause pulmonary circulatory changes either by obstructing vessels or indirectly by releasing vasoactive substances (8, 9).

The purpose of the present study was to investigate central hemodynamics in alcohol or saline pretreated pigs after a standardized soft tissue trauma. A previous study under similar conditions has shown an early platelet trapping (7). Lung mechanics and gas exchange were also elucidated.

Material and methods

Eleven piglets of the Swedish native breed, weighing 25-30 kg, were investigated. The animals were fasted overnight and showed no signs of infection before the study.

Anesthesia

Anesthesia was induced with thiopentone (Pentotal^R, Abbot) 12.5 mg kg⁻¹ intravenously. After oral intubation of the trachea, the animals were mechanically ventilated with a mixture of oxygen and nitrous oxide (FiO₂ 0.5) using a Servo ventilator (Siemens Elema 900B). Anesthesia was then maintained with repeated doses of fentanyl (Leptanal^R, Janssen) and alcuroniumchloride (Alloferin^R, Roche) as needed.

Respiratory rate was set at 20 cycles per minute, inspiration time was 25% and pause time 10% of each cycle. Minute ventilation ($\dot{V}_E$) was adjusted to give an end tidal CO₂ concentration (E'CO₂) between 4.5 and 5.5%. This setting was maintained constant throughout the experiment.

After induction of anaesthesia and orotracheal intubation, the common carotid artery on the left side and the internal jugular veins on both sides were cannulated. A thermodilution catheter (Swan-Ganz 7F) was introduced in the right jugular vein and positioned in the pulmonary artery. These catheters were connected to pressure transducers (Siemens

Elema EMT 34) for recording of aortic pressure (PAo), right atrial pressure (Pra), pulmonary artery pressure (PAP) and pulmonary capillary wedge pressure (PCW), the signals were recorded on an ink jet writer (Siemens Elema EM 81). The thermistor was connected to a digital cardiac output computer (Edwards 9520). 5 ml of saline was injected for determination of cardiac output, the temperature of the injectate was 0-5°C. The mean value of three consecutive determinations of cardiac output was used.

Pressure and volume signals from the ventilator were fed into a calibrated lung mechanic computer (Siemens Elema 940) and read digitally. $\dot{V}_{CO_2}$, carbondioxide elimination ($\dot{V}_{CO_2}$) and effective minute ventilation ($\dot{V}_{E_{eff}}$) were measured using an in-line carbondioxide analyser (Siemens Elema 930) connected to the volume measuring system of the Servo ventilator 900B.

Arterial blood gases were analyzed by conventional methods and corrected for the actual body temperature.

Symbols and abbreviations

b.w.	body weight (kg)
C _{TOT}	total compliance (ml cm H ₂ O ⁻¹)
E'CO ₂	end tidal carbondioxide concentration (%)
h.r.	heart rate (beats min ⁻¹)
PaCO ₂	arterial carbondioxide tension (kPa)
PaO ₂	arterial oxygen tension (kPa)
PAo	mean arterial pressure (mm Hg)
PAP	mean pulmonary pressure (mm Hg)
Pra	mean right atrial pressure (mm Hg)
PVR	pulmonary vascular resistance (units)
PCW	mean capillary wedge pressure (mm Hg)
Q	cardiac output (l min ⁻¹)
SV	stroke volume (ml)
SVR	systemic vascular resistance (units)
SWL	stroke work left ventricle (units)
SWR	stroke work right ventricle (units)
$\dot{V}_{CO_2}$	carbon dioxide elimination (ml min ⁻¹)
$\dot{V}_{E\text{eff}}$	effective minute ventilation (l min ⁻¹)

Calculations

$$\text{PVR} = \frac{\text{PAP} - \text{PCW}}{\dot{Q}}$$

$$\text{SV} = \frac{\dot{Q}}{\text{h.r.}}$$

$$\text{SVR} = \frac{\text{PAo} - \text{Pra}}{\dot{Q}}$$

$$\text{SWR} = \text{SV} (\text{PAP} - \text{Pra})$$

$$\text{SWL} = \text{SV} (\text{PAo} - \text{PCW})$$

Statistics

Current methods for mean, standard deviation and standard error of the mean were used. Evaluation of the results was made with the Wilcoxon Rank-sum test for comparison between the groups and the Friedman test for within group analyses.

Procedure

After induction of anaesthesia and catheterisation a gastric tube was placed in the stomach. In seven animals 15 ml/kg b.w. of 40% ethanol in saline was instilled in the stomach. Four animals received the same volume of saline only. The gastric tube was then removed. Before and 90 minutes after the intragastric instillation, measurements were made and samples for blood gas analyses were taken. The animals were then subjected to a standardized soft tissue trauma against the inguinal region of each hind limb. The trauma consisted of 100 blows of a rubber mallet. Measurements were then made 3, 10 and 30 minutes after trauma.

Results

Central hemodynamics, lung mechanics and gas exchange were unchanged before and 90 minutes after gastric instillation of alcohol or saline. Base excess fell from $+4.8 \pm 1.1$ before to -1.8 ± 1.3 (mean $\pm$ SD) 90 minutes after alcohol was given while it stayed positive in animals who received saline (Table I). The difference in base excess between the two groups 90 minutes after ingestion of alcohol or saline Table I was statistically significant ($p < 0.05$, Table I).

Trauma and circulation

Three minutes after trauma, $\dot{Q}$ decreased in alcohol as well as in saline pretreated animals ($p < 0.01$ and $p < 0.05$ respectively, Fig. 2).

$\dot{Q}$ remained low during the observation period of 30 minutes. There were no differences between the two groups (Fig. 2).

In the alcohol group PVR was increased 3 ($p < 0.05$), 10 (ns) and 30 ($p < 0.05$) minutes after compared to immediately before the trauma (Fig. 3). In the saline group PVR was unchanged. After trauma the difference in PVR between the two groups was statistically significant with the higher values in the alcohol pretreated group (Fig. 3). Before as well as after trauma (Table II) and SWR (Fig. 4) were somewhat higher in the alcohol group. Systemic vascular resistance (SVR) was significantly increased 10 minutes after compared to just before trauma ($p < 0.01$, Fig. 5). SVR was also somewhat increased after trauma in the saline group. There were no differences in SVR between the two groups. SWL was lowered in both groups and remained low up to 30 minutes after trauma (Fig. 6). The reduction of SWL was statistically significant in the alcohol group 3 minutes after trauma ($p < 0.001$, Fig. 6)

- Trauma and ventilation

Total compliance (C_{T0T}) was lower in the alcohol group compared with in the saline group just before as well as after trauma. Between 10 and 30 minutes after trauma C_{T0T} showed a tendency to increase again in the saline group while it continued to decrease in the alcohol group throughout the observation period (Fig. 7). Ten minutes after trauma the difference in PaO_2 was also statistically significant ($p < 0.001$) with the higher values in the saline pretreated group (Table I). $E'CO_2$ and $PaCO_2$ were reduced in both groups 3 and 10 minutes

after trauma and were then increased again in the saline group while it remained low in the alcohol group (Table I). $\dot{V}_{E_{eff}}$ was virtually unchanged in the two groups and $\dot{V}_{CO_2}$ was somewhat decreased in both groups after trauma (Table I).

Discussion

Traffic accidents are far too often associated with alcohol consumption (10) and the influence of alcohol intoxication on the outcome of trauma is important (11). To simulate the alcohol intoxicated patient, an intragastric ingestion of 40% ethanol was used. Blood ethanol concentrations reached by this method, generally 290 mg/100 ml (6), correspond to those found in severely alcohol intoxicated humans. Under these circumstances, the animals in the present study were subjected to a standardized soft tissue trauma.

In the alcohol group, the depth of anesthesia might have been influenced by the pretreatment with ethanol. In these animals the anesthetic level could have been too low resulting in less pain after trauma. However, during the observation period of 90 min, blood pressure and heart rate were similar in the alcohol group compared with the nonalcohol group (table II). There was no difference between the groups in cardiac output (fig 2) and the stroke work of the left heart was decreased to about the same level in both groups (fig 6). These observations indicate that comparable anesthetic levels and hemodynamic conditions in both groups were present at the time of trauma. The only noticeable difference between the two groups was a metabolic acidosis

in the alcohol pretreated animals (tab I). However, this was not believed to have had any significant influence on the reactions to trauma, since pH was stable within normal limits.

In the alcohol pretreated group, PVR was significantly increased after trauma, while it stayed virtually unchanged in the control group (fig III), which apostrofizes the hazardous combination of moderat trauma and alcohol intoxication. Green et al (13) found that high PVR in adult respiratory distress syndrome (ARDS) increased mortality rate. They also found angiographic evidence of pulmonary vascular obstruction as a major event in ARDS. This opinion was emphasized by Zapol et al (12), who from post mortem studies concluded that the increase in PVR in patients suffering from ARDS was caused by obstruction of lung vessles.

The clinical importance of a high PVR in the shock lung syndrome is, however, still debated and its etiology is unclear. In our study, the increase in PVR in the alcohol group was significant already 3 min after trauma. It has recently been shown by Thörne et al (7) that ¹¹¹Indium labelled platelets were trapped in the lungs with an initial maximum 3 min after a soft tissue trauma similar to that used in our study. Furthermore, alcohol is known to change platelet behavior (16) and since no changes in PVR was noted in the control group, the early elevation of PVR was related to the precence of ethanol in the blood. The early pulmonary platelet trapping (PPT) shown by Thörne et al (7) decreased, however, during the observation up to 30 min efter trauma, while in this study PVR remained high during the same length of observation. This suggests that PPT does not continue and it indirectly

suggests that pulmonary platelet plugging of the pulmonary microvasculature is not the only mechanism for the increase in PVR.

Alcohol itself is known to increase PAP (6), which most probably explained the significantly higher PAP in the alcohol group 90 min after ingestion, although PVR at this time before trauma did not differ between the two groups. This alcohol effect on the pulmonary circulation together with other possible mechanisms such as released vasoactive substances from trapped platelets, might have contributed to the higher PVR in the alcohol group.

Thus, a complex interaction between alcohol per se, alcohol affected platelets causing obstruction of the lung vessels as well as vasoconstrictive effects on the lung vessels seems to be a most likely explanation to the initial elevation of PVR as well as to the persistence of high PVR.

In both groups $\dot{Q}$ was significantly reduced after trauma and remained at a low level. From a central circulatory point of view, the most striking difference between the two groups was the continuously high PAP in the alcohol group compared with that found in the saline group. The normal physiological circulatory response to an increase in PAP would have been an increase in the stroke work of the right heart (SWR) which, however, not was found (fig 4). On the contrary, SWR was decreased similarly in both groups during the early phase. This disagrees with the finding of Zapol et al (14) who, in cases of severe ARDS, found that a high PVR was compensated for by an increase in SWR. However, their study was done rather late in the cause of ARDS and one

must assume that their patients were in a circulatory steady state. In our study, no immediate fluid therapy of the traumatic shock was given, a fact that most probably explained the difference in SWR between this series and Zapol et al. Furthermore, alcohol is known to be a myocardial depressant factor which also could have contributed to the absence of response in SWR.

It can be noted in table II that $\dot{V}CO_2$ decreased similarly in both groups. This effect was probably a reflection of the decreased cardiac output. The conformity in the changes of $\dot{V}CO_2$ and $\dot{Q}$ after trauma suggests that no great physiological shunting of blood occurred in the lungs. This is also supported by the high PaO_2 in both groups at all measuring stages, although there was a significantly lower oxygen tension in the alcohol group 10 min after trauma compared with the saline group.

It is concluded that the presence of alcohol in blood results in significantly higher PVR after soft tissue trauma. This is not compensated for by an increase in cardiac work. The mechanism for the development of high PVR is most probably initiated by platelet trapping causing plugging of lung vessels and vasospasm due to the released vasoactive substances. The potentiation of the altered lung circulation by alcohol stresses the negative interaction between trauma and alcohol consumption.

		Before trauma			After trauma		
		0	90		3	10	30
$\dot{V}_{E,eff}$ (l min ⁻¹)	Saline	4,65 $\pm$ 0,39	4,53 $\pm$ 0,39		4,40 $\pm$ 0,33	4,40 $\pm$ 0,40	4,35 $\pm$ 0,47
	Alcohol	5,04 $\pm$ 0,59	4,78 $\pm$ 0,39		4,65 $\pm$ 0,39	4,62 $\pm$ 0,39	4,64 $\pm$ 0,38
$\dot{V}CO_2$ (ml min ⁻¹)	Saline	166,5 $\pm$ 18,1	154 $\pm$ 19,8		134,8 $\pm$ 11,6	138,3 $\pm$ 17,7	139,4 $\pm$ 16,6
	Alcohol	187,1 $\pm$ 15	185,4 $\pm$ 16,1		157,6 $\pm$ 16,4	156,7 $\pm$ 12,9	147,6 $\pm$ 15,8
$\dot{E}O_2$ (%)	Saline	4,63 $\pm$ 0,25	4,13 $\pm$ 0,3		3,92 $\pm$ 0,17	3,81 $\pm$ 0,31	4,0 $\pm$ 0,27
	Alcohol	4,67 $\pm$ 0,3	4,78 $\pm$ 0,28	*	4,15 $\pm$ 0,25	4,17 $\pm$ 0,22	3,88 $\pm$ 0,29
PaCO ₂ (kPa)	Saline	4,12 $\pm$ 0,27	4,28 $\pm$ 0,14	*	3,86 $\pm$ 0,15	3,86 $\pm$ 0,10	4,33 $\pm$ 0,2
	Alcohol	4,20 $\pm$ 0,33	4,58 $\pm$ 0,2	**	3,70 $\pm$ 0,08	3,96 $\pm$ 0,11	3,86 $\pm$ 0,09
PaO ₂ (kPa)	Saline	28,5 $\pm$ 1,15	32,6 $\pm$ 1,2	*	26,9 $\pm$ 2,3	31,5 $\pm$ 1,8	30,7 $\pm$ 1,4
	Alcohol	21,9 $\pm$ 3,5	27,1 $\pm$ 1,43		23,5 $\pm$ 2,7	27,1 $\pm$ 0,72	25,2 $\pm$ 1,02
pH	Saline	7,58 $\pm$ 0,02	7,57 $\pm$ 0,01		7,50 $\pm$ 0,02	7,57 $\pm$ 0,01	7,51 $\pm$ 0,02
	Alcohol	7,53 $\pm$ 0,02	7,41 $\pm$ 0,02		7,48 $\pm$ 0,03	7,42 $\pm$ 0,03	7,41 $\pm$ 0,37
BE	Saline	7,2 $\pm$ 1,8	8,40 $\pm$ 1,1		7,5 $\pm$ 1,2	5,1 $\pm$ 0,9	3,7 $\pm$ 0,4
	Alcohol	4,8 $\pm$ 1,1	-1,8 $\pm$ 1,3	**	-1,0 $\pm$ 2,0	-3,7 $\pm$ 1,74	3,6 $\pm$ 2,4

TABLE I Mean values ($\pm$ SEM) of $\dot{V}_{E,eff}$, $\dot{V}CO_2$, PaCO₂, PaO₂, pH and Base Excess (BE) in alcohol and saline pretreated animals before and after trauma. Asterix above the figure indicates significant difference between alcohol and saline pretreated animals. Asterix between the figures indicates significant difference between to the previous value in the same group. * p<0,05, ** p<0,01, *** p<0,001

		Before trauma			After trauma		
		0	90		3	10	30
PAO (mmHg)	Saline	151 [±] 16	124 [±] 25, 5		109, 5 [±] 20, 7	107, 3 [±] 17, 7	113, 0 [±] 6, 2
	Alcohol	147, 4 [±] 15, 5	129, 7 [±] 21, 4	*	90, 5 [±] 16, 7	** 108, 3 [±] 16, 2	104 [±] 19
PAP (mmHg)	Saline	17 [±] 2, 8	14, 5 [±] 2, 9		9, 0 [±] 2, 7	10, 4 [±] 9, 1	11, 1 [±] 4, 1
	Alcohol	21, 4 [±] 5, 9	24, 1 [±] 4, 8		24, 7 [±] 7, 8	19, 6 [±] 3, 3	21, 9 [±] 4, 8
Pra (mmHg)	Saline	-1, 9 [±] 0, 5	2, 5 [±] 1, 2		0, 5 [±] 0, 35	0, 8 [±] 0, 4	-0, 6 [±] 1, 8
	Alcohol	6, 0 [±] 2, 6	6, 1 [±] 1, 9		3, 6 [±] 1, 9	3, 0 [±] 1, 5	3, 0 [±] 1, 8
PCW (mmHg)	Saline	-9, 0 [±] 3, 6	8, 3 [±] 3, 1	*	4, 1 [±] 2, 1	5, 1 [±] 2, 4	4, 4 [±] 2, 9
	Alcohol	7, 6 [±] 1, 2	10, 1 [±] 2, 1		8, 8 [±] 2, 4	8, 8 [±] 1, 7	6, 9 [±] 1, 9

TABLE II Mean values ([±]SEM) of PAO, PAP, Pra, and PCW before and after trauma. Asterix above the figure indicates significant difference between alcohol and saline pretreated animals. Asterix between the figures indicates significant difference compared to the previous value in the same group. * p<0,05, ** p<0,01, *** p<0,001

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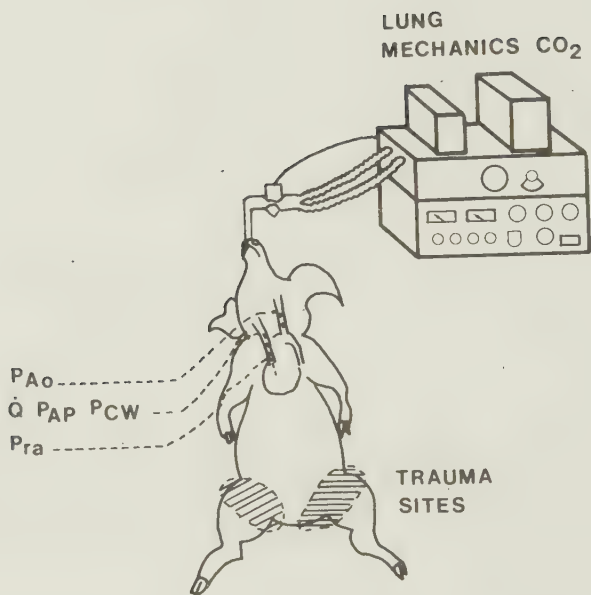


Fig 1. Schematic presentation of measured variables and the ventilator equipment used. The sites for the blunt tissue trauma are also indicated.

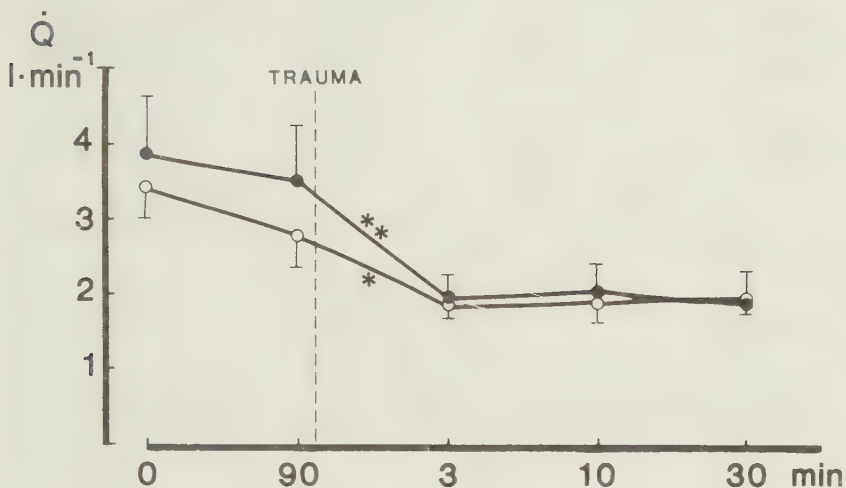


Fig II. Mean values ($\pm$ SEM) of $\dot{Q}$ before and 90 min after ingestion of alcohol (filled symbols) and saline (open symbols) as well as 3, 10 and 30 min after trauma.

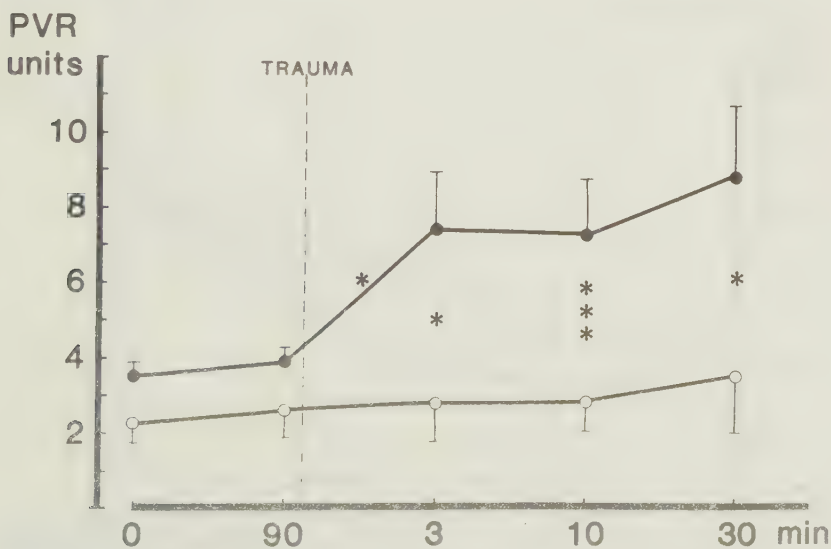


Fig III. Mean values ($\pm$ SEM) of PVR before and 90 min after ingestion of alcohol (filled symbols) and saline (open symbols) as well as 3, 10 and 30 min after trauma.

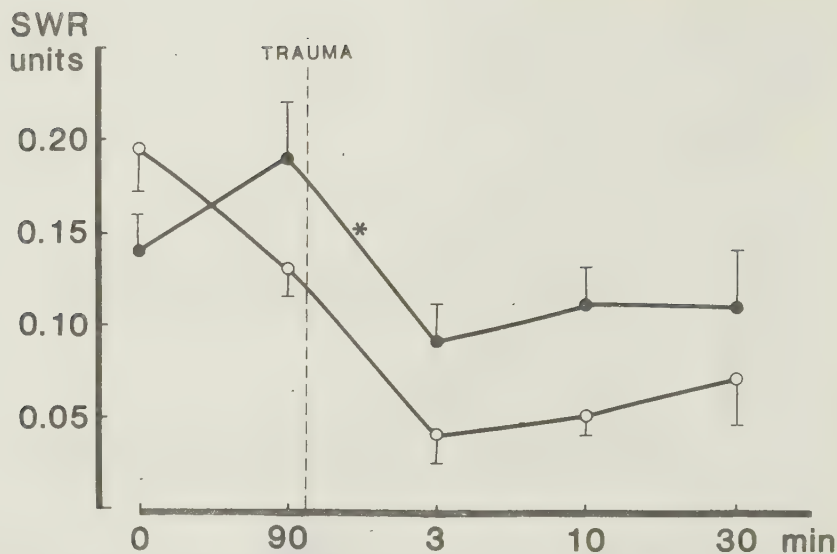


Fig IV. Mean values ($\pm$ SEM) of SWR before and 90 min after ingestion of alcohol (filled symbols) and saline (open symbols) as well as 3, 10 and 30 min after trauma.

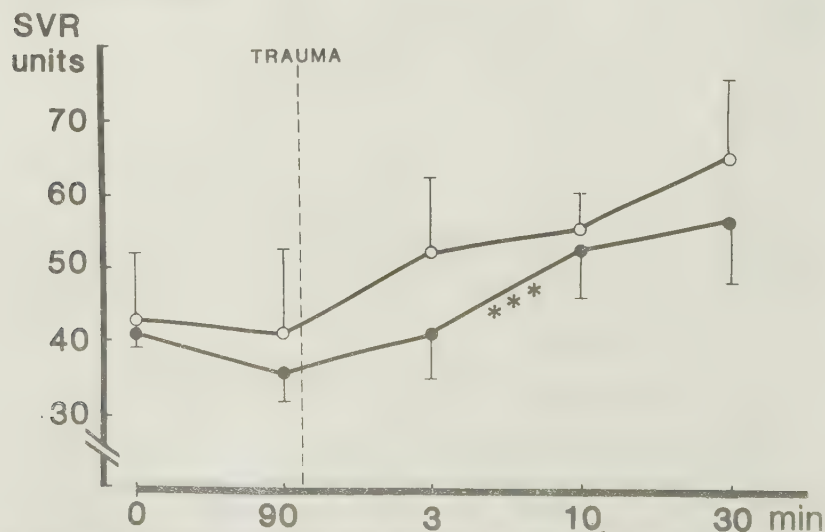


Fig V. Mean values ($\pm$ SEM) of SVR before and 90 min after ingestion of alcohol (filled symbols) and saline (open symbols) as well as 3, 10 and 30 min after trauma.

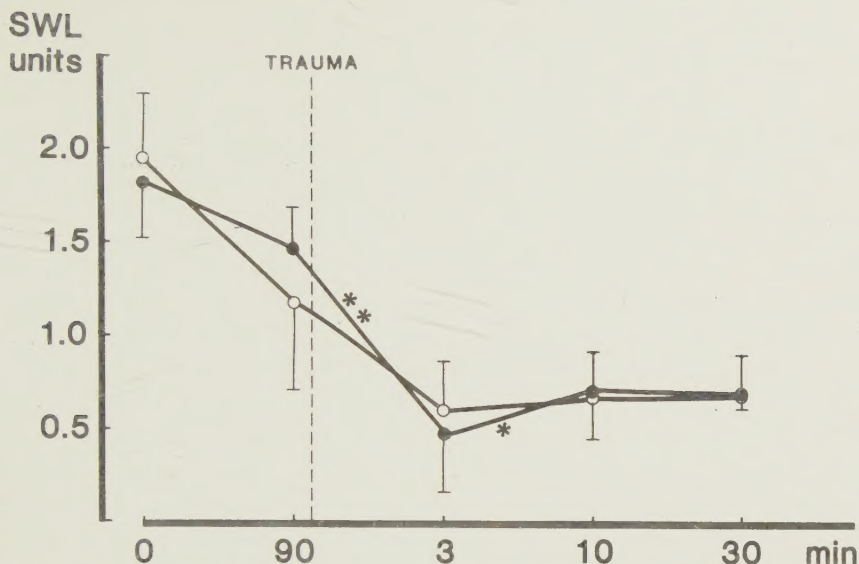


Fig VI. Mean values ($\pm$ SEM) of SWL before and 90 min after ingestion of alcohol (filled symbols) and saline (open symbols) as well as 3, 10 and 30 min after trauma.

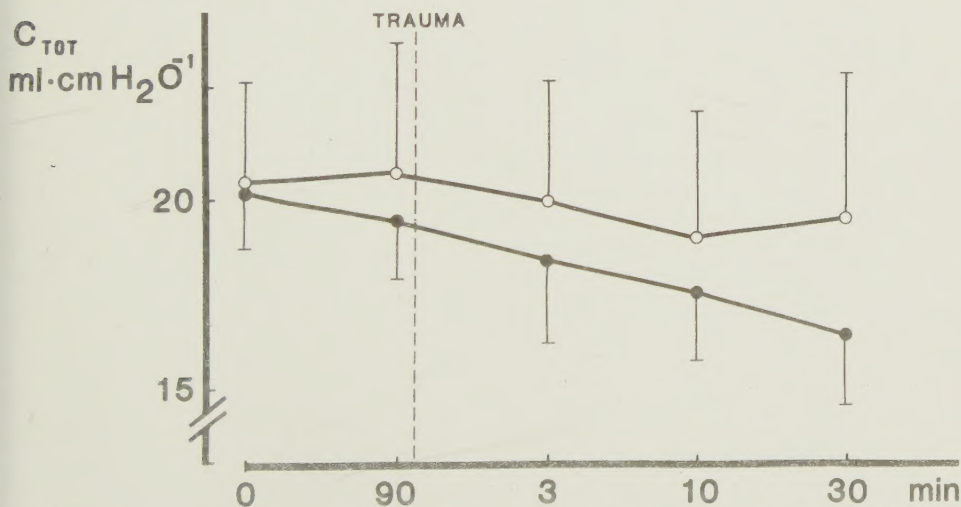


Fig VII. Mean values ($\pm$ SEM) of C_{TOT} before and 90 min after ingestion of alcohol (filled symbols) and saline (open symbols) as well as 3, 10 and 30 min after trauma.

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